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An Investigation of filter-feeding in the tilapia *Oreochromis niloticus (L.)*.

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Mark E. Northcott, BSc.

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To my Family

The experimental system Bangkok, Thailand.

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"The silence that accepts merit as the most natural
thing in the world, is the highest applause."

Emerson, *Nature*

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Declaration

This is to certify that Mr M.E. Northcott carried out the experimental work and produced the scientific content and composition of the joint paper entitled :

"The development and structure of the pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*)"

by M.E. Northcott and M.C.M. Beveridge (*J. Zool., Lond.* : 215, 1988).

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Erratum

For pharyngeal pad / pads read pharyngeal bone / bones.

List of Tables

Table		Page
1	Relation of mucus cell dimensions (μm) and goblet mucus cell concentration on the gill arch to fish standard length (mm). Figures in parenthesis are ± 1 S.D.	20
2	Composition of BGII algal growth medium.	60
3	One-way ANOVA of mean algal biovolume concentrations of experimental treatments.	61
4	Student's *t* values for initial versus final algal biovolume concentrations of experimental treatments.	67
5	Model I ANOVA of *A. cylindrica* colony length in the two size class feeding experiments.	67
6	Tukey multiple comparison test for *A. cylindrica* colony length in the two size class feeding experiments.	68
7	Variance ration, F and students *t*-test for *A. cylindrica* colony length before and after grazing.	69
8	*O. niloticus*. Data for regression equations ($\log_{10} y = a + b\log_{10}x$ for 85 mm S.L. fish and $y = a + b\log_e x$ for the 40 mm S.L. fish) of ingestion rate (y, $\mu m^3\ g^{-1}\ h^{-1}$) on mean treatment biovolume concentration (x, $\mu m^3\ ml^{-1}$).	70
9	Phytoplankton biovolume data.	103
10	Zooplankton biovolume data.	104
11	Comparison of estimated and calculated volume contributions.	104
12	Percentage volume composition of the diet of 80 mm S.L. *O. niloticus* at 1200 hours, 7.11.87.	110
13	Stomach Fullness Indices, SFI; stomach content dry weight, SCD (mg) and stomach pH during the second diurnal feeding study on 80 mm S.L. *O. niloticus*.	111
14	Percentage biovolume composition of the diet of 80 mm S.L. *O. niloticus* at 2100 hours, 9.11.87.	112
15	Range of SFI's and percentage volume composition of the diet of 35 mm S.L. *O. niloticus* during the diurnal feeding trial.	113
16	Percentage volume composition of the diets of four size classes of *O. niloticus*.	114
17	Genera of phytoplankton found in the diet of the four size classes of *O. niloticus*.	115
18	Data from sample weighings.	115

List of Figures

Figure		Page
1	The relation between gill raker number and fish standard length.	21
2	The relation between gill raker width and spacing and fish standard length.	22
3	(a) Diagram showing the general structure of a gill arch from the tilapia, *Oreochromis niloticus*. Gill arch from the left side of the pharyngeal 'basket'.	23
	(b) Diagram of transverse section of a gill arch.	24
4	Diagram of the denticle showing the four regions apparent from histological and SEM studies.	25
5	Diagram of the recirculating aquaria used in the filter feeding trials of *O. niloticus* on blue-green algae.	62
6	Diagrams of (a) *A. cylindrica* and (b) *M. aeruginosa* showing the dimensions measured for biovolume calculations.	63
7a-d	The relationship between ingestion rate, I ($\mu m^3 g^{-1} h^{-1}$) and the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3 ml^{-1}$) for the two size classes of *O. niloticus* feeding on the two species of blue-green algae.	71
8	Multiple plot of the ingestion rate, I ($\mu m^3 g^{-1} h^{-1}$) versus the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3 ml^{-1}$).	73
9a-d	The relationship between filtration rate, F (ml $g^{-1} h^{-1}$) and the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3 ml^{-1}$) for the two size classes of *O. niloticus* feeding on the two species of blue-green algae.	74
10a-d	Combined plots of ingestion rate, I ($\mu m^3 g^{-1} h^{-1}$) and filtration rate, F (ml $g^{-1} h^{-1}$) versus the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3 ml^{-1}$).	76
11a-e	Percentage frequency histograms for *A. cylindrica* colony length in the experimental feeding chambers before and after grazing by 40 mm S.L. *O. niloticus*.	78
12a-d	Percentage frequency histograms for *A. cylindrica* colony length in the experimental feeding chambers before and after grazing by 85 mm S.L. *O. niloticus*.	81
13	The relationship between empty stomach dry weight (mg) and fish wet weight (g).	105
14a-d	Daily chlorophyll 'a' (mg m^{-3}) and pH values in the cages and ponds.	116
15a-c	Dissolved oxygen, D.O. (mg l^{-1}) and temperature (°c) values during the diurnal feeding trials.	118

		Page
16	Percentage volume composition of the diet of 80 mm S.L. *O. niloticus* at 1200 hours, 7.11.87.	120
17	The relationship between stomach pH and stomach contents dry weight (mg).	121
18	Percentage volume composition of the diet of 35 mm S.L. *O. niloticus* during the diurnal feeding trial.	122
19	Percentage volume composition of the diets of four size classes of *O. niloticus*.	124
20	Ivlev's electivity indices for the four size classes of *O. niloticus*.	126

List of Plates

Plate		Page
1	Scanning Electron Micrograph (SEM) of the second gill arch from a 65 mm S.L. fish.	26
2a-d	SEM study of gill raker development on the second gill arch.	27
3a-d	SEM study of the microbranchiospine development on the second gill arch.	29
4	SEM of the microbranchiospine from a 64 mm S.L. fish showing the ridged surface epithelium.	31
5	SEM showing division of microbranchiospines.	31
6a-c	Histological development of the gill rakers from transverse sections of the gill arch. Alcian Blue / PAS.	32
7	Transverse section of microbranchiospine from a 165 mm S.L. fish. Alcian Blue / PAS x 400.	34
8	Longitudinal section of microbranchiospine from a 78 mm S.L. fish. Alizarin Red x 400.	34
9	Longitudinal section of microbranchiospine from a 165 mm S.L. fish. Alcian Blue / PAS x 400.	35
10	SEM of denticle showing hollow mid-section.	35
11a-c	SEM study of upper pharyngeal pad development.	36
12a-c	SEM study of lower pharyngeal pad development.	38
13	Close up of lower pharyngeal pad from a 14 mm S.L. fish.	40
14	Transverse section of one half of the upper pharyngeal pad from a 36 mm S.L. fish. Alcian Blue / PAS x 100.	41
15	Transverse section of part of the lower pharyngeal pad from a 36 mm S.L. fish. Alcain Blue / PAS x 100.	41
16	SEM of a sheet of mucus entrapped algae caught on the gill rakers of a 65 mm S.L. fish.	83
17a-c	Close ups of th e mucus entrapped algae.	83
18	SEM of mucus entrapped algae on the lower pharyngeal pad of a 65 mm S.L. fish. Gill arch IV is visible on the left of the micrograph.	85
19	SEM close up of a pharyngeal tooth and associated mucus covering from the lower pharyngeal pad of a 65 mm S.L. fish.	85

Abbreviations used in plates.

ac	*Anabaena cylindrica* colony
afg	anterior face of gill arch
b	hollow base of denticle
cmc	clavate mucus cell
d	denticle
dt	denticle tip
ga	gill arch
gb	gill raker bud
gf	gill raker face
gk	gill raker keel
gmc	goblet mucus cell
gr	gill raker
ibp	inner boundary of pharyngeal pad
lc	loose connective tissue
m	mid-section of denticle
mas	mucus entrapped algal sheet
mc	mucus cell
mcp	mucus covering of pharyngeal tooth
mf	mucus filament
mn	mucus network
ms	microbranchiospine
p	bony plate
pt	pharyngeal tooth
rse	ridged surface epithelium
se	surface epithelium
ste	stratified epithelium
t	taste-bud
tb	tooth-bud
ts	tooth-socket
vt	vascularised tissue.

List of Appendices

		Page
Appendix 1	Water quality, feeding ecology and histological details of the pharyngeal mucus cells of *O. niloticus* from the ponds at Tinsulanonda Songkhla Fisheries College, Songkhla, S. Thailand.	145
Appendix 2	Histological details of the pharyngeal mucus cells of *Oreochromis (Alcolapia) alcalicus grahami* (Boulenger) from Lake Magadi and Lake Nakuru, Kenya.	146
Appendix 3	Estimation of the ingestion rate of 35 mm S.L. *O. niloticus* during the diurnal feeding trial at A.I.T., Bangkok, Thailand.	148
Appendix 4	Published paper.	149

Contents

Page

Abstract 1

Chapter 1 Introduction 3

Chapter 2 Histology and ultrastructure 8

2.1 Introduction 8

2.2 Materials and Methods 10

2.2.1 Fish 10
2.2.2 Gill Raker Morphometrics 10
2.2.3 Scanning Electrom Microscopy (SEM) 11
2.2.4 Histology 11

2.3 Results 13

2.3.1 Gill Raker Morphometrics 13
2.3.2 Surface Ultrastructure of the gill arch 13
2.3.3 Fine Structure of the gill arch 15
2.3.4 Surface ultrastructure, Development and Histology of the Pharyngeal pads 18

2.4 Discussion 42

Chapter 3 Quantitative study of filter feeding 50

3.1 Introduction 50

3.2 Materials and Methods 53

3.2.1 Algal Culture 53
3.2.2 Experimental System 54
3.2.3 Experimental Protocol 54
3.2.4 Sample Analysis 55
3.2.5 Determination of Ingestion and Filtration rates 57
3.2.6 SEM study of the phytoplankton entrapment 59

3.3 Results 64

3.3.1 Ingestion and Filtration rate experiments 64
3.3.2 SEM study of the phytoplankton entrapment 66

3.4 Discussion 86

Page

Chapter 4 Thailand Fieldwork 92

4.1 Introduction 92

4.2 Materials and Methods 96

4.2.1 Experimental Design 96
4.2.2 Project Investigations 97
I General Water Quality 97
II Diurnal Trials 97
III Ontogeny of Feeding apparatus and Habits 98
4.2.3 Sampling Protocol 98
I Sampling Technique 98
II Stomach Content Collection 98
III Tissue Fixation 99
IV Stomach pH 99
V Dry weight of stomach contents 99
4.2.4 Sample Analysis 100
I Water Samples 100
II Stomach Samples 101

4.3 Results 106

4.3.1 Water Quality 106
4.3.2 Diurnal Feeding Studies 106
4.3.3 Ontogeny of Feeding habits 107
4.3.4 Histology and Ultrastructure of the pharyngeal feeding apparatus 108
4.3.5 Fish Growth 109

4.4 Discussion 129

Chapter 5 Discussion 138

Appendices 145

References 168

Abstract

Various aspects of the feeding ecology of the tilapia, *Oreochromis niloticus* (L.), were investigated. An ultrastructural and histological survey of the pharyngeal apparatus associated with filter feeding, in laboratory-held fish, demonstrated that the development was complete by approximately 40 mm S.L. The filter apparatus would appear not to work as a passive sieve and the presence of large numbers of mucus cells substantiate a theory of mucus entrapment of algae. Three mucosubstances were demonstrated to be produced by the pharyngeal apparatus. Neutral and sialyated mucins were produced by the mucus cells of the gill rakers and were proposed to be involved in the filtering process. The mucus cells of the tooth sockets in the pharyngeal pads produced a sulphated mucosubstance and this was proposed to act as a 'heavy' lubricant during the raking action of the pads. A SEM study revealed ultrastructural differences in the mucus related to function.

A quantitative investigation of filter feeding revealed the ingestion and filtration rate dynamics of *O. niloticus* grazing on two species of blue-green algae, *Anabaena cylindrica* and *Microcystis aeruginosa*. Both 40 mm and 85 mm S.L. fish were demonstrated capable of filter feeding on the above phytoplankton. The results substantiated a universality in the fundamental regulatory mechanisms of suspension feeders.

During fieldwork in Thailand the feeding ecology of small (≈ 35 mm S.L.) and large (≈ 80 mm S.L.) *O. niloticus* was investigated. The results demonstrated the diverse dietary habits of both size classes of fish, including detritivory, herbivory and carnivory. The proposed switch in diet at approximately 60 mm T.L., from omnivorous, particulate feeding to phytoplanktivorous filter feeding, was shown not to be an obligate event. The ultrastructural and histological investigation revealed similar findings to the laboratory-held *O. niloticus*. The histological results indicate continual mucus production from the

pharyngeal apparatus and some other mechanism must be capable of preventing ingestion.

In conclusion, the work has elucidated further the feeding mechanism of *O. niloticus* and demonstrated the diverse feeding habits of both small and large fish.

Chapter 1

Introduction

"Let us go into the Sea of Cortez, realizing that

we become forever a part of it."

Steinbeck, *The Log from the Sea of Cortez.*

The tilapias are cichlid fishes of Africa and the Levant that have become dispersed throughout the warm countries of the world for fish-farming. The family Cichlidae is widely distributed in Africa (including Madagascar) and Palestine, in South and Central America, in southern India and in Sri Lanka (Lagler *et al.*, 1977). Trewavas (1983)* described extensively the mouth-brooding tilapia, placing the species into three genera, *Sarotherodon*, *Oreochromis* and *Danakilia*. Substrate-spawning species, belonging to the genus *Tilapia*, were not considered.

The potential of tilapia as good aquaculture species is widely accepted and must be due, in part, to the fact that the majority of cultured tilapias feed as micro- or macro- herbivores and detritivores (Balarin & Hatton, 1979). However, they are also capable of utilizing an enormously wide variety of the natural foods available in tropical waters (Bowen, 1982). Infact, Philippart & Ruwet (1982) stated that a comparison of feeding in the same species of tilapia in a large range of water bodies reveals a very great variability of feeding regime. This is an element of the remarkable plasticity and ecological adaptability of tilapias.

Present knowledge of the feeding mechanisms and dietary habits of phytoplanktivorous tilapia species has come from a large number of studies which demonstrate the great variability in feeding ecology within and among such species.

* The taxonomic classification of Trewavas (1983) is used throughout this thesis.

Weatherly (1972) stated that it may be relatively simple to infer the general nature of a fish's food from a knowledge of its fundamental morphology. Lazzaro (1987) proposed that the evolution from macrophagy to microphagy is marked by the development of specialised structures, including elaborate gill rakers. The use of intricate gill rakers in the filter feeding of planktivorous fishes has been well documented (e.g. Dz. de Ciechomski, 1967; Shen, 1969; Iwata, 1976; King & MacLeod, 1976; Hossler *et al.*, 1979; O'Connell, 1981; Rosen & Hales, 1981). However, Suyehiro (1942) noted that a number of families contain planktivorous species that possess very coarse gill rakers. The simple form of the gill rakers in phytoplanktivorous tilapia species has been observed by many researchers (Fish, 1951; Greenwood, 1953; Whitehead, 1959; Trewavas, 1983).

Numerous researchers have considered gill rakers as passive sieves (Durbin & Durbin, 1975; Rosen & Hales, 1981; Drenner *et al.*, 1984a; Mummert & Drenner, 1986). Whilst this mechanism would appear feasible for species with elaborate, comb-like rakers, comparable to the filtering appendages of many zooplankton species (Nival & Nival, 1976), the gill raker morphology of *Oreochromis niloticus* (Linnaeus) does not appear to represent an efficient passive device due to their comparatively small size and wide spacing. Indeed, Fryer & Iles (1972) described the feeding apparatus of tilapias as simple and unspecialised. However, various workers have recently disputed that even the sieve-like mesh of the zooplankton filtering appendages actually act as passive sieves. Koehl & Strickler (1981) observed that the feeding appendages of copepods behaved as 'paddles' rather than 'sieves'. Parcels of water containing particles were captured and the water removed by squeezing it out (Paffenhöfer *et al.*, 1982; Strickler, 1982).

La Barbera (1984) states that virtually all suspension feeders capture particles from the water at low Reynolds numbers with cylindrical filtering elements. The Reynolds number (R_e) is the ratio of inertial to viscous forces for a flow situation:

$$R_e = \frac{\rho v h}{\mu}$$

where ρ is the density, v is the relative velocity of a fluid across a solid object and h is a linear dimension of the object and μ the dynamic viscosity of the fluid. In a situation where the water flow around an element occurs at a low Reynolds number the flow is laminar and viscous forces dominate rather than inertial forces. Reynolds numbers less than 1 are considered representative of laminar conditions. Gerristen & Porter (1982) demonstrated that the surface chemistry of both particles and filtering appendages is important for *Daphnia magna* feeding at low Reynolds numbers. Likewise, Braimah (1987) found increased filtering efficiency in immature *Simulium bivattatum* and *Isonychia campestris* bearing elaborate sieve-like filtering appendages indicating adhesion of particles to filtering structures probably by hydrophilic-hydrophobic or ionic interaction.

Exceptions involving the use of cylindrical filtering elements include anuran tadpoles (Wassersug & Rosenberg, 1979) and larval lamprey (Mallat, 1981). However, Wassersug & Rosenberg (1979) estimated that flow near the surface of the branchial food traps would occur at low Reynolds numbers, although they stated that the structure of the food traps do not lend themselves to a simple hydrodynamic analysis. Friedland (1985) estimated that the Reynolds number for the flow regime around the branchiospinules (secondary projections of the gill rakers) of the Atlantic menhaden, *Brevoortia tyrannus*, would be 2-3. Therefore, the flow would be in a transitional state of turbulence.

Greenwood (1953) proposed mucus entrapment of phytoplankton in the filtering feeding mechanism of *Oreochromis esculentus*. Sinha (1975) stated simplistically that herbivorous species, such as *Labeo rohita*, required large quantities of mucus for trapping and transporting food particles and, therefore, had large numbers of mucus cells in the

bucco-pharyngeal region. Carnivorous species, by contrast, such as *Clarias batrachus*, requiring less mucus had a sparse occurrence of mucus cells. Mucus entrapment mechanisms in suspension feeders have been proposed for other fish species (Robotham, 1982; Friedland, 1985; Sibbing & Uribe, 1985), for larval lamprey (Mallatt, 1981) and numerous anuran tadpoles (Savage, 1952; Kenny, 1969a,b; Wassersug, 1972).

Despite the diversity of filter feeders and, indeed, proposed filtering mechanisms in aquatic animals Seale *et al.* (1982) proposed a universality in the fundamental regulatory mechanisms of suspension feeding. This statement was made in view of the similar feeding responses of invertebrate and tadpole suspension feeders.

The results of quantitative filter feeding studies have been used to create functional response models. A number of curves (rectilinear, Ivlev, Michaelis-Menten) have been fitted to data from filter feeding studies on zooplankton (Mullin *et al.*, 1975); tadpoles (Seale & Wassersug, 1979; Seale & Beckvar, 1980; Seale *et al.*, 1982) and fish (Ivlev, 1961; Drenner *et al.*, 1987). The Michaelis-Menton curve was fitted to results for filter feeding *Sarotherodon galilaeus* (Drenner *et al.*, 1987) although inert particles and not natural food items were used in the trials. Infact, Mullin *et al.* (1975) demonstrated statistically that all three curves could be justifiably fitted to the zooplankton data. Despite much work on the functional response of filter feeding zooplankton and anuran tadpoles little data exists on filter feeding fish species.

The phytoplanktivorous diet of large *O. niloticus* is well documented (Fryer & Iles, 1972; Moriarty & Moriarty, 1973; Trewavas, 1983; Getachew, 1987). Several workers have noted an ontogenetic shift in the diet and feeding modes for *O. niloticus* (Yashouv & Chervinski, 1961). Moriarty *et al.* (1973) observed a switch from 'pecking' at plant and animal material to 'gulping' water containing suspended phytoplankton. Such observations were summed up by Trewavas (1983) who stated that, in the natural habitat,

the feeding mode and diet of the tilapia, *O. niloticus*, changes from omnivorous particulate feeding, when young fry, to phytoplanktivorous filter feeding by 60 mm total length (≈ 50 mm standard length).

However, the contradictory results of several workers suggest that the switch in diet and feeding mode appears not to be an obligate event. Yashouv & Chervinski (1960) found that *O. niloticus* were capable of ingesting a wide spectrum of prey items available in the pond. Pantastico *et al.* (1982) found good growth of *O. niloticus* fry (initial weight ≈ 0.012g) feeding solely in suspensions of phytoplankton including the species *Chlorella ellipsoidea*, *Nannochloris* sp. and *Scenedesmus quadricauda*. Considering the microscopic size of the algae the fish must have been 'pump' filter feeding, despite being well below the standard length at which the diet shift is reported to take place in the wild. Lazarro (1987) defined 'pump' filter feeders as those fish using rhythmic suctions to capture prey items, while swimming slowly, or remaining quite stationary.

In view of the above information the aim of this thesis was to conduct a detailed investigation of various aspects of filter feeding in *O. niloticus* which would result in a clearer picture of the feeding ecology of this species. An ultrastructural and histological investigation of the strucutres associated with filter feeding is described, the results of which, it was hoped, would help determine the mechanism of filter feeding. Secondly, a quantitative study of filter feeding on phytoplankton was undertaken in an attempt to generate possible functional response curves. Finally, an ontogenetic study was made of the feeding ecology of *O. niloticus* held in the same environment, during the developmental stage at which their diet is reported to change in the natural habitat, in an effort to elucidate whether the 'switch' in diet was in fact a consequence of a change in functional morphology or in environmental resources.

Chapter2

Histology and ultrastructure

2.1 Introduction

In the natural habitat, the feeding mode and diet of the tilapia *O. niloticus* changes from omnivorous particulate feeding, when young fry, to phytoplanktivorous filter feeding by 60 mm total length (Trewavas, 1983). Although the phytoplanktivorous diet of *O. niloticus* is well documented (Fryers & Iles, 1972; Moriarty & Moriarty, 1973; Trewavas, 1983), the mechanisms by which filter feeding takes place is poorly understood.

A qualitative and quantitative assessment of the role of gill rakers in filter feeding in fishes has been made by Hyatt (1979). Many workers have considered gill rakers as passive sieves (Durbin & Durbin, 1975; Rosen & Hales, 1981; Drenner *et al.*, 1984). Whilst this method of filter feeding seems feasible in species with elaborate, comb-like rakers, such as the anchovies (*Engraulis anchiota, E. japonica, E. capensis*), pilchard (*Sardinops ocellata*), paddle fish (*Polyodon spathula*) and goldfish (*Carassius auratus*) (Dz. de Ciechomski, 1967; Shen, 1969; Iwata, 1976; King & MacLeod, 1976; Rosen & Hales, 1981), the gill raker morphology of *O. niloticus* does not appear to represent an efficient passive filtering device, as the gill rakers are comparatively small and widely spaced.

Rubenstein & Koehl (1977) state that the presence of mucus-covered elements in biological filters is evidence that 'aerosol' feeding, in which particles are attracted to a sticky filter, occurs. However, this theory remains largely untested and to date aerosol suspension feeding has been demonstrated only in the brittle star, *Ophiopholis aculeata* (La Barbera, 1978). In other animals, such as anuran and lamprey larvae, mucus is purported to play some role in food entrapment (Savage, 1952; Kenny, 1969a, b; Wassersug, 1972; Mallat,

1981), whilst in the Atlantic menhaden, *B.tyrannus*, Friedland (1985) has proposed that particulate food is primarily captured by mechanical sieving, although its transport could be mucus mediated.

In tilapias, too, theories of filter feeding remain largely speculative. Greenwood (1953) has suggested that suspended phytoplankton is drawn into the buccal cavity where it becomes entrapped with copious quantities of mucus, although this has yet to be demonstrated.

The possible role of microbranchiospines in filter feeding has also been suggested. Microbranchiospines ('micro gillrakers') are small dermal ossifications located in the epidermis, forming a continuous row close to the base of the gill arch. The wide spread presence of microbranchiospines in the Cichlidae has been interpreted as a synomorphy uniting the family (Stiassny, 1981). Similar structures have also been found in a number of the lower percoids, including *Lates* (Greenwood, 1976), *Genes* and *Haemulon* (Stiassny, 1981). The term 'microbranchiospine' has sometimes been confused with 'branchiospinule' and 'microspine', terms applied by Monod (1961) and Iwata (1976) to the secondary projections found on the gill rakers of the Atlantic menhaden, and many other cyprinadae.

Microbranchiospines were first described in the tilapias *Tilapia zillii* and *O. niloticus* by Gosse (1956). Their function, however, is still a matter of debate. The involvement of microbranchiospines in filter feeding was suggested by Gosse (1956) and promulgated by a number of other workers (Whitehead, 1959; Bone & Marshall, 1982; Drenner *et al.*, 1984b). However, this theory has been disputed by others due to the lack of functional analysis and the fact that microbranchiospines occur in a wide range of cichlids with diverse feeding habits. Moreover, they are not solely restricted to planktivorous species (Fryer & Iles, 1972; Stiassny, 1982; Trewavas, 1983).

Posterior to the gill arches in *O. niloticus* are dorsal and ventral toothed pharyngeal pads. Ebeling (1957) suggested that the pharyngeal teeth may be involved in the actual filtering process of eastern Pacific mullets. However, Greenwood (1953) and Fryer & Iles (1972) suggest that the function of the pads in *Oreochromis* species is simply to rake the mucus-entrapped food particles into the oesophagus.

It was, therefore, decided to study the pharyngeal apparatus associated with filter feeding in *O. niloticus*. The study was achieved by detailed ultrastructural and histological investigations. The structure and morphology of the filtering apparatus was examined in a wide range of fish size classes covering the size at which *O. niloticus* is stated to switch its feeding habit and mode in the natural habitat. The results of such an ontogenetic study would be used in an attempt to clarify possible feeding mechanisms.

2.2 Materials and Methods

2.2.1 Fish

Specimens of *O. niloticus* examined were derived from genetically homogenous stock held at the Institute of Aquaculture (McAndrew and Majumdar, 1983).

2.2.2 Gill Raker Morphometrics

Buccopharyngeal feeding structures, the gill arches and upper and lower pharyngeal pads, were carefully removed from the fish using a stereo-zoom dissecting microscope. Gill arches used for the measurement of gill raker number, width and spacing were examined fresh. Following a preliminary examination of the gill arch apparatus which revealed that apart from the outer gill rakers of gill arch I and the inner rakers of arch IV, the structure and size of the rakers was very similar, data was collected from the outer row of rakers of

gill arch II. For fish of standard length (S.L.) ≤ 35mm the dissected gill arch was placed on a microscope slide and the rakers enumerated by counting the bony gill raker bars using a light microscope. With this method it was possible to obtain data from fish as small as 8mm S.L. The gill rakers of fish ≥40mm S.L. were counted using the stereo-zoom microscope. The gill raker widths and spacing, of fish 14 to 68mm S.L. were measured using a calibrated ocular graticule and light microscope. Ten measurements of the above parameters per fish were taken from the middle section of the gill arch II.

2.2.3 Scanning Electron Microscopy (SEM)

The ultrastructure and development of the feeding apparatus was studied using an ISI - 60A scanning electron microscope. Intact pharyngeal apparatus or individual gill arches and pharyngeal jaws were rinsed briefly in distilled water before being placed in freshly prepared 2.0% paraformaldehyde and 2.5% glutaraldehyde in a 0.1M cacodylate - HCl, 2mM calcium chloride buffer (pH 7.4). After at least two hours fixation at 4°C the material was rinsed with several changes of buffer. Specimens were then fixed for at least 2 hours at 4°C in buffered 1% osmium tetroxide. They were then dehydrated through a graded acetone series, stored in 70% acetone at 4°C if necessary, and critical-point dried in a Polaron dryer before being mounted on brass stubs with silver conducting paint and coated with a layer of gold in an Edwards S150 sputter coater.

2.2.4 Histology

Complete heads or dissected gill arches were fixed in 10% neutral buffered formalin, processed and embedded in wax. Longitudinal and transverse sections were made of individual gill arches. Transverse sections were taken from complete heads of fish ≤ 40mm S.L. All sections were 5μm in thickness.

Sections were stained with Haematoxylin-eosin (H.E.) for structural analysis or Alcian blue (pH 1.0 or 2.5)/Periodic Acid Schiff (P.A.S) for identification of the mucus cell

glycoproteins (see Drury and Wallington, 1980, for details). All sections were examined using a light microscope.

Examination of the sections stained with alcian blue (pH 1.0 or 2.5)/PAS showed that there were two distinct types of mucus cell present in the gill arches; large, clavate cells and small, superficial goblet cells. Thirty measurements for each cell type were made from each fish. Cells were measured in 6-10 sections per animal. The clavate cells were measured using the longest axis.

The density of the small mucus cells was estimated from the anterior face of the gill arch. The number of cells in a 250µm length of epithelium was counted. Cells were counted in 8-10 sections per animal.

An accurate assessment of the density of the large mucus cells was not attempted.

In order to determine the structural composition of the microbranchiospines, longitudinal and transverse 5µm serial sections were stained with Wiegart and Van Gieson, Alizarin Red, Von Kossa and Mallory Heidenhain. Staining was also performed on similar sections to assess whether the microbranchiospines might play some role in mechanoreception. The stains were specific for nervous tissue, Palmgren's silver impregnation method, with and without toning step, and a modified Weil haematoxylin method for myelin (see Drury & Wallington, 1980, for details of above staining methods).

2.3 Results

2.3.1 Gill Raker Morphometrics

Fig. 1 shows the development of gill raker number in fish of 8 to 87mm S.L. The maximum number, 28, of gill rakers on the arch is reached at 50-60mm S.L. The relationship between fish standard length and gill raker number is ($Y = 6.537 \log_e X + 0.173$; $r = 0.98$).

Fig. 2 shows the change in gill raker width and spacing in fish of 14 to 68mm S.L. For each fish analysed the gill rakers were wider than the inter-raker spacing. The relationships between fish standard length and gill raker width ($Y = 114.542 \log_e X - 257.568$; $r = 0.98$) and standard length and inter-raker spacing ($Y = 52.037 \log_e X - 101.481$; $r = 0.94$) are also both exponential.

2.3.2 Surface Ultrastructure of the gill arch

A preliminary examination of the pharyngeal region revealed that although there were slight differences in structure, the surface features of the four gill arches were very similar. Each gill arch supports two rows of gill filaments and two rows of gill rakers (Fig. 3a, b). Gill arch I differed from the other arches in that the outer rakers were more elongate forming the boundary with the operculum. The inner row of rakers had the same structure as those of arches II and III and the outer row of rakers of gill arch IV. Gill arch IV differed in that it was much shorter, laterally widened and the inner, elongate rakers overlapped each other with no or very few inter-raker spaces. The inner rakers of gill arch IV formed the boundary with the lower pharyngeal jaw.

The location and distribution of the microbranchiospines was revealed following an SEM examination of the gill arches. They occurred as a single row of toothed projections,

tapering towards their tips and directed towards the adjacent gill rakers. They occurred consistently on the external faces of gill arches II, III and IV, but were also found in greatly reduced numbers on the internal faces of these arches in the same fish. The microbranchiospines were distributed as a continuous row on the external faces, but on the internal faces were found in greatest number on gill arch IV, and tended to occur as a group, most often in the middle of the arch.

Plate 1 shows gill arch II from a fish of 65mm S.L. illustrating the general features. It is apparent that material fixed as described is free of mucus. At high power it was very difficult to find mucus cell pores, which suggests that this procedure fixes them in the closed state. All surfaces of the gill arch and rakers are covered with a pavement epithelium that has concentric surface microridges. A feature of the gill arch is the presence of the 'club'- like gill rakers.

Gill arch development was studied in fish from 9mm S.L. upwards (Plate 2a-d). The gill rakers of fish 9-10mm S.L., (Plate 2a) consist of small outgrowths composed of 2-4 taste-bud papillae. The anterior leading face of the arch is covered in rows of taste-buds that connect opposite rakers on the arch. The gill rakers of fish 14-18mm S.L. (Plate 2b) had further developed into more prominent outgrowths bearing about 10-14 taste-buds.

The gill rakers had also started to differentiate a posterior trailing section, termed the 'keel', and an anteriorly directed leading face. The gill arch face is covered in rows of taste-buds. Plate 2c & d illustrate further development of the gill rakers. The 'mature' structure of the raker is reached at about 40mm S.L. and is shown in Plate 2d. The gill raker has a smooth trailing keel that is continuous with the side of the gill arch. The two leading edges of the gill raker each carry a single row of taste-buds and the face contains a multiple row that extends onto the anterior face of the gill arch. The occurrence of taste-buds in rows on the face of the gill arch decreases in fish of 40mm S.L. upwards.

Using the SEM , the development and proliferation of the microbranchiospines was also studied. No microbranchiospines were apparent in fish smaller than 9mm S.L. Irregularly spaced mounds of tissue, covered with ridged epithelium were observed on the gill arches of fish 11-13mm S.L. in the region where microbranchiospines are found in older fishes. Microbranchiospine rudiments with denticles were consistently observed in fishes of 15mm S.L., although they were often poorly developed, each rudimentary microbranchiospine consisting of a mount of tissue with perhaps one denticle projecting through the ridged epithelium. In fish of 19-21mm S.L., the microbranchiospine rudiments had further developed, although their morphology and spacing was very irregular (Plate 3a).

On the gill arches of fish 23mm S.L., the microbranchiospines had developed into a row of regularly shaped and spaced structures (Plate 3b). The close abutment of neighbouring microbranchiospines is clearly shown in the gill arches of large fish in Plate 3d. Concentric surface microridges were apparent from SEM preparations (Plate 4).

On numerous occasions, and in fish of all sizes, microbranchiospines were found to be in the course of dividing. Plate 5 shows a section of a gill arch in which the process is apparent. Division of the main body of the microbranchiospine proceeds from the base and is followed by the proliferation of new denticles along the inner faces.

2.3.3 Fine Structure of the gill arch

The staining of sections with alcian blue (pH 1.0 or 2.5)/P.A.S. revealed the presence of two distinct types of mucus cell associated with the gill arches; small, superficial cells that were typically goblet shaped and large, deeper penetrating cells that were clavate. The small mucus cells stained either red or a combination of red/blue following alcian blue (pH 2.5)/P.A.S. treatment and contained either neutral or a mixture of neutral/acidic glycoproteins respectively. They stained red after treatment with alcian blue(pH1.0)/P.A.S. The large, clavate mucus cells stained blue following alcian blue (pH 2.5)/P.A.S.and faint

pink following alcian blue (pH 1.0)/P.A.S.indicating a major predominance of acidic mucosubstances. No mucus cells stained blue after alcian blue (pH 1.0)/P.A.S. Therefore there were no sulphated mucosubstances associated with gill arch or rakers.

The gill rakers were covered with a compact squamous epithelium that overlies a stratified epithelium of 35 to 60µm thickness in fish of 40-200mm S.L. A transverse section of a gill raker, Plate 6a, shows that the epithelium of the anterior face contains many taste-buds as observed using SEM, and a few mucus cells. A detailed examination of the material revealed that the large mucus cell occurred only in association with the trailing keel of the gill raker, Plate 6a. The epithelium of the keel also contained the small type mucus cell in reduced numbers. The thick, stratified epithelium of the gill raker stands on a basement membrane of about 2.5µm, thickness. The centre of the raker is filled with a loose, vascularised connective tissue and contains a single bony gill raker element, observed in whole mounts of fish from 8mm S.L. upwards. The epithelium covering the anterior face and sides of the gill arch is considerably thinner, 8-12µm, and contains only small type mucus cells and few taste-bud papillae.

A study of gill arch development as observed from histological preparations revealed several interesting features. The mucus cells of fish of about 14mm S.L. were small, goblet type, and there were no large, clavate cells associated with the gill raker buds. The large mucus cells first became apparent in fish of 18mm S.L. However, in fish 18-22mm S.L. the number of acidic cells associated with the gill rakers was low and only occurred towards the posterior of the feeding chamber. In fish of 24mm S.L. there is an increase in the number of clavate mucus cells associated with the keel of the gill raker and an increase in the size (Table 1). At this time there is also an increase in the general occurence of the large mucus cells so that by about 36mm S.L. they occur in all gill rakers on all gill arches.

A quantitative estimation of large mucus cells in the keel of the gill rakers was not attempted. However, the increase in number with increasing fish size is apparent from

Plate 6a-c.

Table 1 shows the relationship of various mucus cell parameters to fish standard length. There is a noticeable increase in size of the clavate mucus cells although there is only a slight increase in the diameter of the goblet mucus cells with increasing fish standard length. There was a significant difference ($P < 0.001$) between the dimensions of large clavate and small goblet mucus cells in all fish examined. The concentration of goblet mucus cells on the gill arch increases in fish up to about 26mm S.L. There were significant differences between the mucus cell concentration of fish 14mm S.L. and fishes of 26-168mm S.L. ($t = 3.91$; d.f. = 18; $P < 0.05$) and between fishes of 18 and 168mm S.L. ($t = 2.30$; d.f. = 18; $P < 0.05$).

The microbranchiospines are embedded in the epidermal layer which covers the gill arch. The main body of each microbranchiospine consists of eosinophilic, acidophilic, bony material (Plate 7) which bears numerous lateral, tusk-like denticles, projecting forwards towards the gill rakers, and inwards towards the surface of the gill arch (Plate 8). The microbranchiospines are covered by a thin layer of epithelial tissue which extends over the denticles. Occasional mucus cells and eosinophilic granular cells were observed in the epithelium at the base of the microbranchiospines (Plate 9).

The bony plate and denticles sit 50-100μm above the gill arch on a base of epithelial and connective tissue underlain by a matrix of well-vascularised tissue in which collagen fibres are present (Plate 7). The denticles are suprisingly complex structures and can be described as comprising of four distinct regions: a base of chondroblast and osteoblast cells which extend into the main body of the microbranchiospine, a mid-section, partially hollow, which consists of bone, and a tip which appears to be covered in a sheath (Plate 8 & 9). A discontinuity layer is apparent in some preparations and seems to separate the denticle base from the rest of the structure. The hollow nature of the middle section of the denticles is confirmed from examination of a number of denticles which had becomed detached during

preparation for SEM (Plate 10), although bone stem cells are apparent in the lumen from light microsopy preparations (Plate 7).

The denticles' tips and terminal sheath, which often appeared to protude through the surface epithelium (Plate 4), proved impossible to stain, even after decalification. Both methods for the demonstration of calcium salts gave a positive result, although due to the orange-red birefringent precipitate of the alizarin stain, it was unsuitable for fine staining of the denticles. The Von Kossa method resulted in the mid-section and tip staining black and a similar uniform staining of these sections was also visible after treatment with Mallory Heidenhain Casson, and Weigerts Van Geison, the colours being blue and pink/yellow respectively. However, with Haematoxylin and Eosin, and Alcian Blue (pH 2.5)/P.A.S., it was possible to differentiate a distal region of the denticle. Both procedures stained the base of the denticle and the main body of the microbranchiospine pink,the denticle mid-section remaining white following Haemotoxylin and Eosin, and blue following Alcian blue/P.A.S. treatment.

The structure of the denticle is summarized diagrammatically in Figure 4.

The results of the staining specific for nervous tissue were as follows. With Palmgren's stain, the microbranchiospines appeared brown/black on either a yellow/brown or grey background, depending on whether or not the toning step was used. The modified Weil haematoxylin method stained all tissues other than red blood cells and eosinophilic granular cells light brown in colour. No nervous tissue was observed in any of the preparations.

2.3.4 Surface ultrastructure, Development and Histology of the Pharyngeal pads

Plates 11a-c and 12a-c show the development of upper and lower pharyngeal pads respectively. It can be seen that there is a proliferation in the number of teeth between 14

and 24mm S.L. Development of the pharyngeal pads with respect to shape and tooth density appears complete at 40mm S.L.

The epithelium between the teeth has surface-ridges and on the lower pharyngeal pads of fish about 14mm S.L. there were many taste-buds. These taste-buds were not borne on papillae and did not, therefore, project far above the level of the neighbouring epithelial cells (Plate 13). The occurence of the tastebuds on the pads decreases in fish of 14mm S.L. upwards as the number of teeth increased.

The histology of both upper and lower pharyngeal pads is shown in Plates 14 and 15. The inner boundary of both pads is formed by a layer of compact squamous cells, on top of which lies a zone of connective tissue interdispersed with irregular bone formation. Above this layer is a region of loose connective tissue which contains many tooth buds in various stages of development.

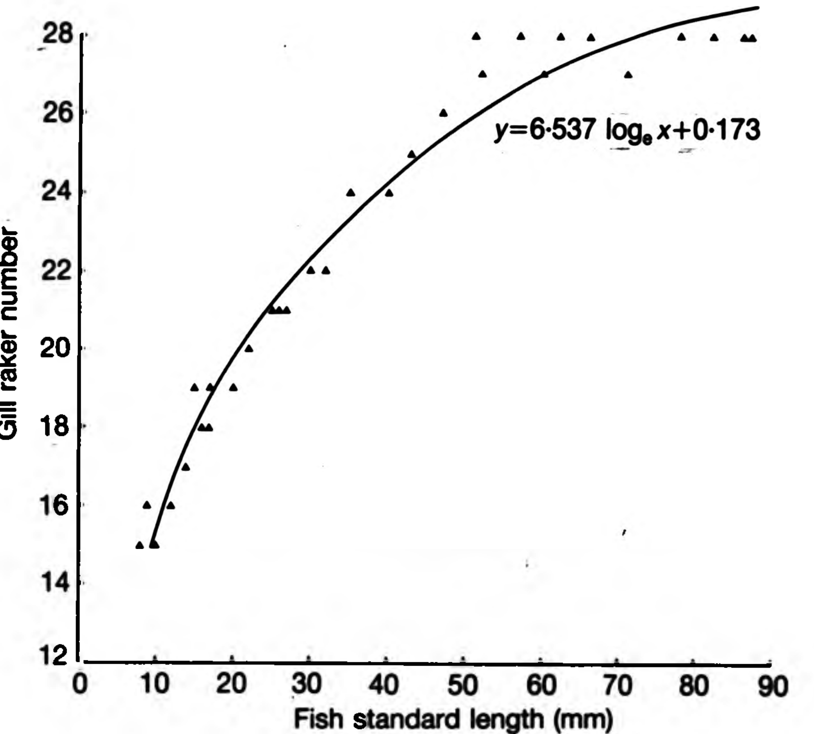
In the middle of the pharyngeal pads is a layer of irregularly structured bone on top of which are hinged the mature teeth. The tooth hinge is constructed of fibrillar material. The teeth project above the stratified squamous surface epithelium of the pharyngeal pads via tooth sockets that are lined with large spherical mucus cells. The diameter of the cells was $14.99 \pm 1.22\mu m$ in a fish 36mm S.L. These cells stain blue following alcian blue (pH 1.0)/P.A.S. and, therefore, contain a sulphated glycoprotein. The mucus cells of the pharyngeal pads were less common in fish 14-22mm S.L. but were numerous in fish of ≥ 36 mm S.L. During histological sectioning and staining of whole heads it was noted that there were sulphated mucus producing cells lining the sockets of the mandibular teeth.

The brown coloured caps of the pharyngeal teeth, visible in fresh material were absent following decalcification.

Table 1. Relation of mucus celll dimensions (μm) and goblet mucus cell concentration on the gill arch to fish standard length (mm). Figures in parenthesis are ± 1 S.D.

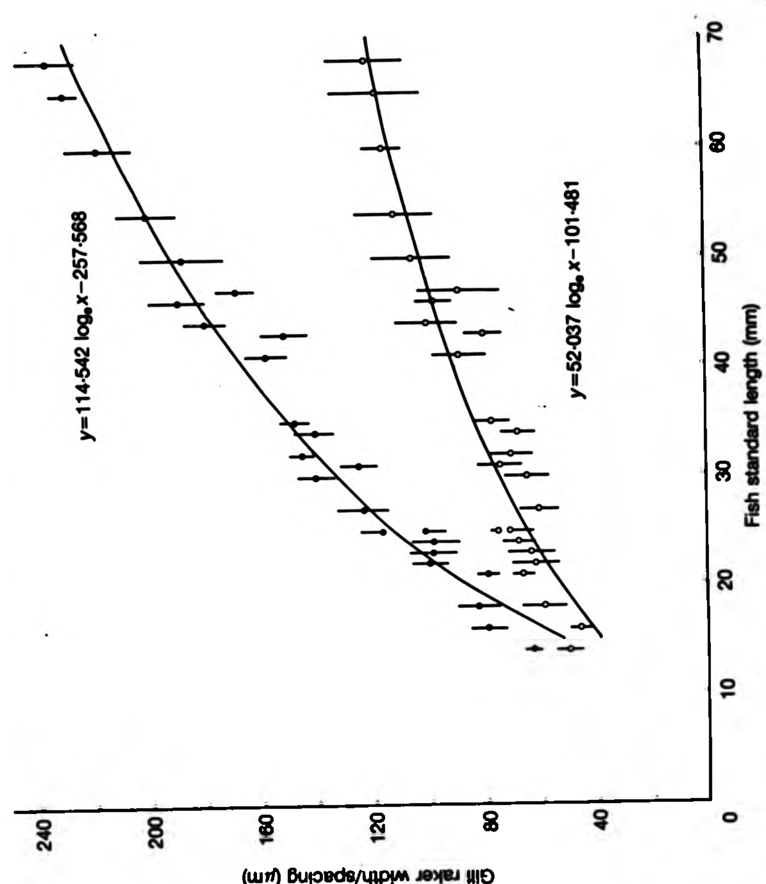
Fish Standard length (mm)	Mucus cell dimensions (μm)		Goblet cell concentration (cells) 250 μm epithelium
	Clavate	Goblet	
14	—	9.93(1.01)	11.50(2.46)
14	—	9.10(1.23)	—
18	15.97(1.36)	9.23(1.59)	13.90(2.51)
22	16.30(1.83)	9.89(1.30)	14.50(2.92)
24	19.43(3.28)	9.97(1.42)	—
26	19.60(3.30)	10.47(1.29)	16.70(3.40)
33	23.42(2.14)	10.64(1.20)	—
36	28.49(2.36)	10.55(1.44)	16.90(2.47)
41	26.10(2.89)	11.13(1.45)	—
62	30.38(4.17)	12.03(1.65)	16.88(3.68)
77	31.45(3.21)	12.44(2.12)	17.13(3.36)
103	31.54(3.04)	12.03(1.25)	16.90(3.45)
168	33.88(4.25)	11.95(1.42)	17.30(3.95)

Fig. 1. The relation between gill raker number and fish standard length



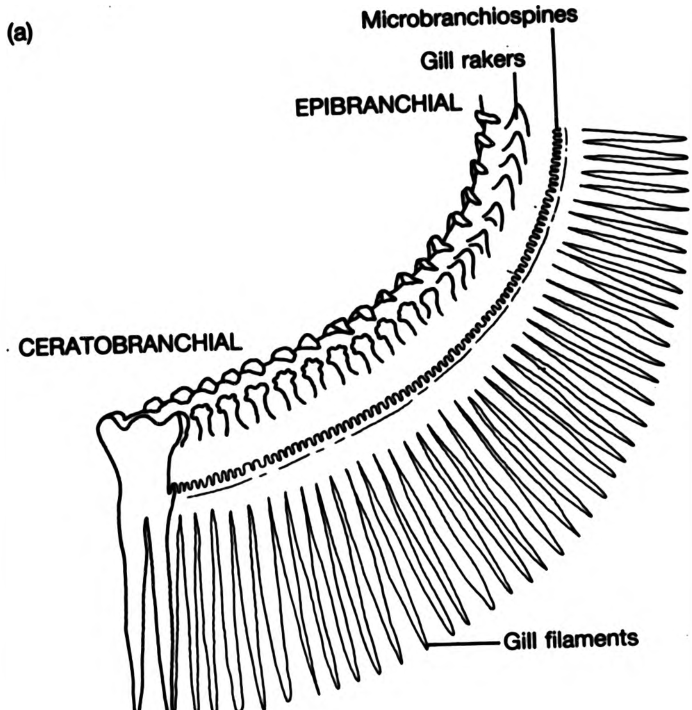
28
26
24
22
20
18
16
14
12
Gill raker number
0
10
20
30
40
50
60
70
80
90
Fish standard length (mm)
$y=6{\cdot}537 \log_e x+0{\cdot}173$

Fig. 2. The relation between gill raker width (●) and spacing (○) and fish standard length (mean ± 1 S.D.).



y=114·542 log$_e$ x−257·568
y=52·037 log$_e$ x−101·481
Gill raker width/spacing (μm)
Fish standard length (mm)
0
10
20
30
40
50
60
70
40
80
120
160
200
240

Fig. 3. (a) Diagram showing the general structure of a gill arch from the tilapia, *Oreochromis niloticus*. Gill arch from the left side of the pharyngeal 'basket'. (b) Diagram of transverse section of a gill arch.


(a)
Microbranchiospines
Gill rakers
EPIBRANCHIAL
CERATOBRANCHIAL
Gill filaments

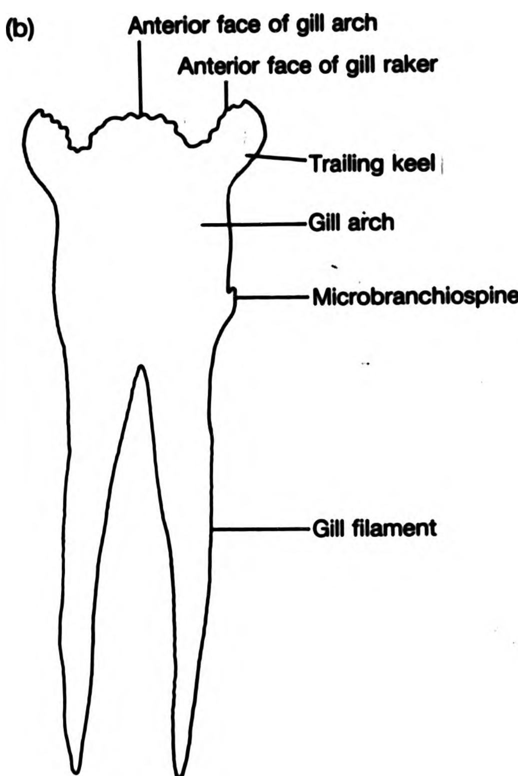

(b)
Anterior face of gill arch
Anterior face of gill raker
Trailing keel
Gill arch
Microbranchiospine
Gill filament

Fig. 4. Diagram of the denticle showing the four regions apparent from histological and SEM studies. 1. terminal 'sheath'; 2. tip; 3. main body of denticle with lumen; 4. base of denticle and area of ankylosis; 5. main body of microbranchiospine.

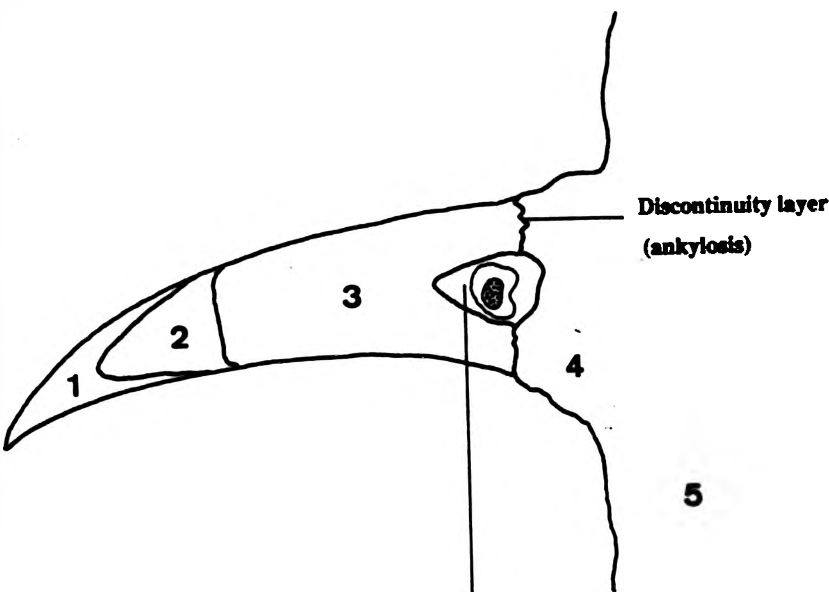
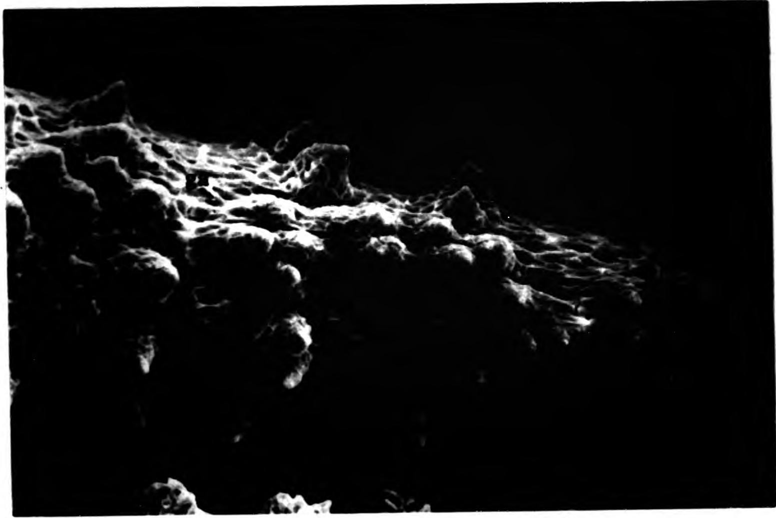

Discontinuity layer
(ankylosis)
1
2
3
4
5
Lumen,bone stem cells present

Plate 1. Scanning Electron Micrograph (SEM) of the second gill arch from a 65 mm S.L. fish.

300 µm

Plate 2. SEM study of gill raker development on the second gill arch. (a) 9 mm S.L. fish; (b) 16 mm S.L. fish; (c) 40 mm S.L. fish; (d) 100 mm S.L. fish.

(a)

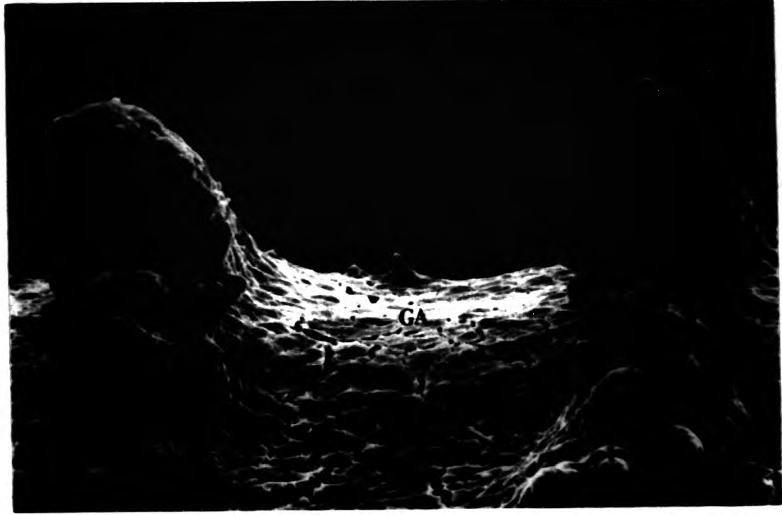


40 µm

(b)

40µm

(c)



(d)

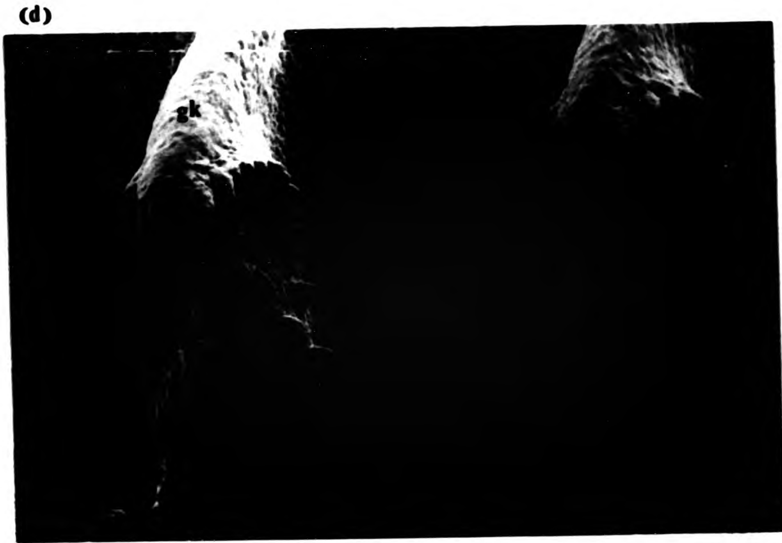
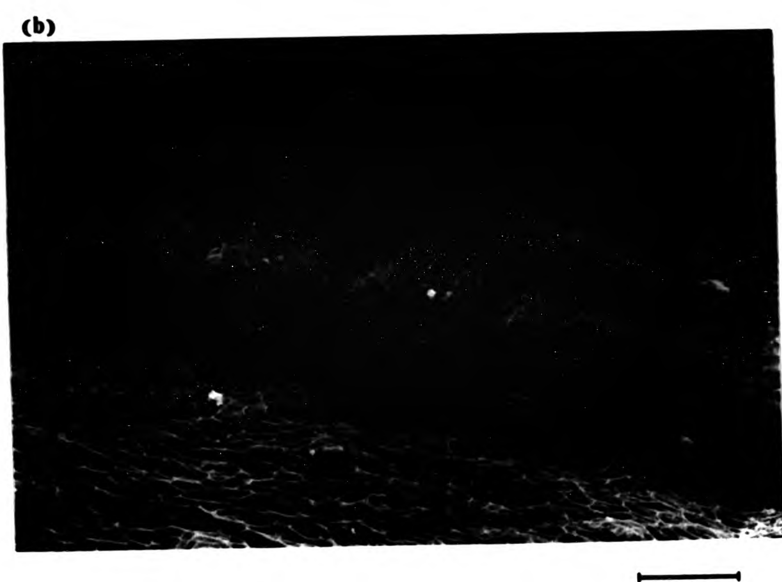


Plate 3. SEM study of microbranchiospine development on the second gill arch. (a) 21 mm S.L. fish; (b) 25 mm S.L. fish; (c) 64 mm S.L. fish; (d) 180 mm S.L. fish.

(a)

(b)

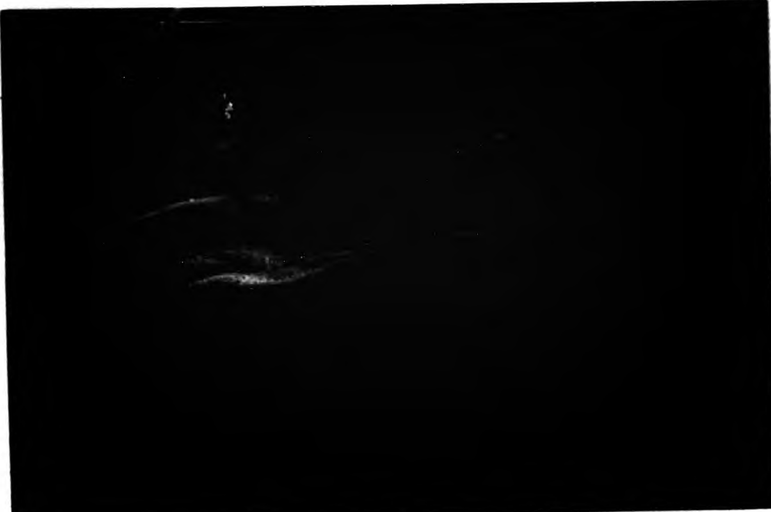


(c)

(d)

Plate 4. SEM of microbranchiospine from a 64 mm S.L. fish showing the ridged surface epithelium.

Plate 5. SEM showing division of microbranchiospines.


10 µm

d
20µm

Plate 6. Histological development of the gill rakers from transverse sections of the gill arch. Alcian Blue / PAS. (a) 61 mm S.L. fish x 200; (b) 22 mm S.L. fish x 400; (c) 78 mm S.L. fish x 200.

(a)

(b)

(c)

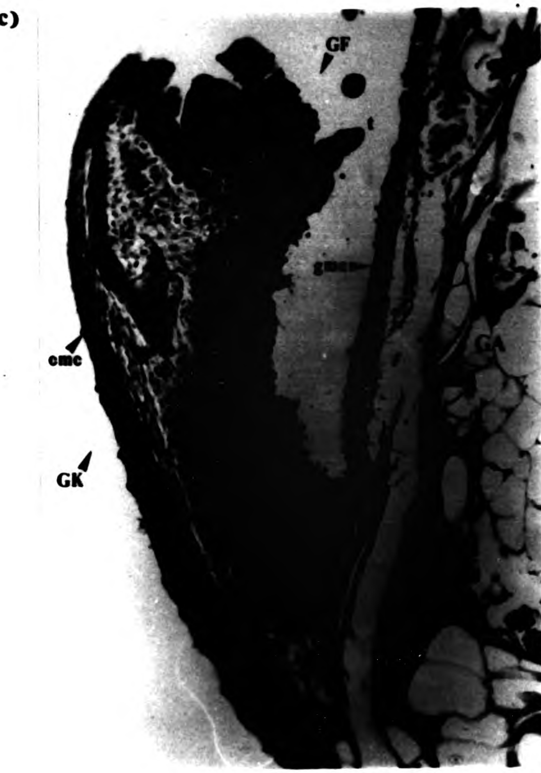


Plate 7. **Transverse section of microbranchiospine from a 165 mm S.L. fish. Alcian Blue / PAS x 400.**

Plate 8. **Longitudinal section of microbranchiospine from a 78 mm S.L. fish. Alizarin Red x 400.**

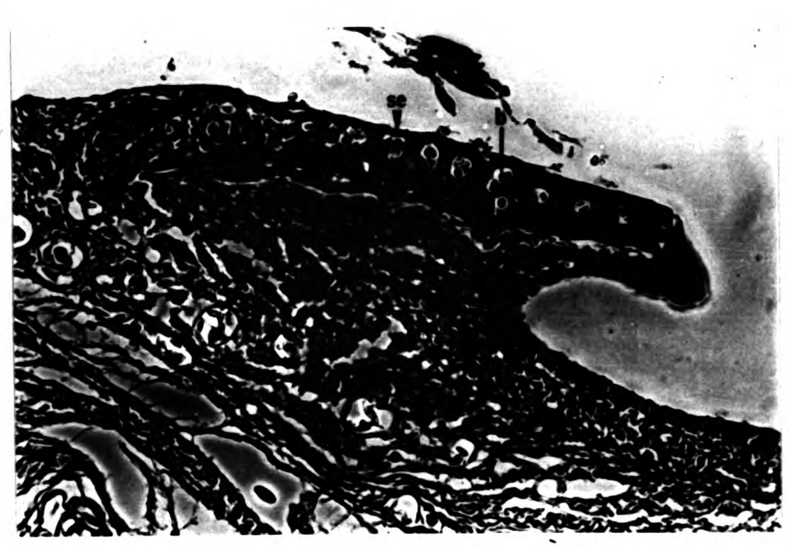


Plate 9. Longitudinal section of microbranchiospine from a 165 mm S.L. fish. Alcian Blue / PAS x 400.

Plate 10. SEM of denticle showing hollow mid-section.

5µm

Plate 11. SEM study of upper pharyngeal pad development. (a) 14 mm S.L. fish; (b) 24 mm S.L. fish; (c) 57 mm S.L. fish.

(b)

(c)

400 µm

Plate 12. **SEM study of lower pharyngeal pad development. (a) 14 mm S.L. fish; (b) 24 mm S.L. fish; (c) 40 mm S.L. fish.**

(a)

(b)

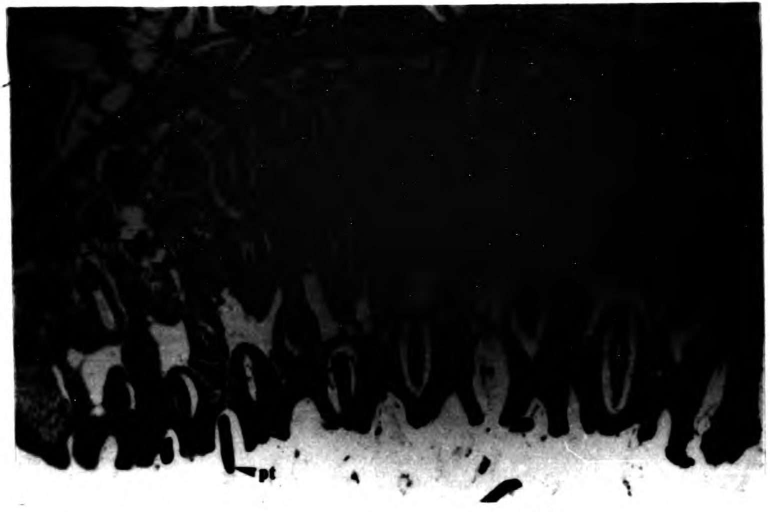
(c)

Plate 13. Close up of lower pharyngeal pad from a 14 mm S.L. fish

10 µm

Plate 14. Transverse section of one half of the upper pharyngeal pad from a 36 mm S.L. fish. Alcian Blue / PAS x 100.

Plate 15. Transverse section of part of the lower pharyngeal pad from a 36 mm S.L. fish. Alcian Blue / PAS x 100.


pt

ts

2.4 Discussion

This study has provided a description of the surface and histological features of the pharyngeal structures associated with filter feeding in *O. niloticus* of between 9mm and 200 mm S.L., covering both the particulate and filter feeding periods of their life histories.

Microbranchiospines were observed in fish as small as 9mm S.L. Examination by both light and electron microscopy revealed then to be bony, plate-like structures with partially hollow extensions (denticles), covered by a thin epithelial layer. The terminal sheath over the denticles (see Fig. 4) proved histochemically inactive to any of the stains used. It appears not to be composed of enameloid, as this substance is removed after decalcification, a method used prior to all but the calcium-specific treatments. The main body of the denticles (Regions 2 and 3, Fig 4) contains calcium salts and is composed of the same bony material as the main body of the microbranchiospine (Region 5, Fig. 4). However, the differential staining of the distal region (Region 2, Fig. 4) with Haematoxylin and Eosin and Alcian Blue/P.A.S. suggests that it has a slightly different structure, being P.A.S. negative and acidic. This may be important in the attachment of the terminal sheath. The presence of a discontinuity layer and ankylosis of the structure to the main body of the microbranchiospine (Region 4, Fig. 4) is characteristic of a Type I tooth attachment mode in actinopterygian fishes as described by Fink (1981). However, although mature denticles have a similar morphology to teeth (Orvig, 1967; Shaeffer, 1977) there are a number of inconsistencies, in the presence of a terminal sheath and in the absence of any enameloid-staining regions.

In this study the microbranchiospines were observed to be attached to the gill arch by a solid base of connective and epithelial tissue. They thus formed a series of blind-ending channels opening out towards the inhalent respiratory current, rather than a continuous gutter, as previously described (Gosse, 1956; Whitehead, 1959). The microridges observed on the microbranchiospine surface, gill arch and gill rakers have been observed on the surfaces of

other teleost gill structures (Hughes & Wright, 1970; Olsson & Fromm, 1973; Kendal & Dale, 1979; Hossler *et al.*, 1979; Hughes, 1979; Mattey *et al.*, 1980). The pattern and dimensions of the whorls are similar to those observed by Kendal & Dale (1979) on the gill rakers of trout. These microridges probably function in anchoring mucus to the various gill surfaces (Hughes, 1979) including the microbranchiospines.

Unfortunately the results of this study do not reveal the function of the microbranchiospines. Several workers have suggested their involvement in filter feeding (Gosse, 1956; Whitehead, 1959; Bone & Marshall, 1982; Drenner *et al.*, 1984b). However, Drenner *et al.* (1987) found that surgical removal had no effect on either particle ingestion rates or selectivity. Moreover, a number of tilapia species which have no microbranchiospines and comparatively few gill rakers utilise planktonic algae as a source of food, although, as pointed out by Trewavas (1983), these species occur in alkaline, soda lakes, where the algae tend to be long chain species (e.g. *Spirulina*) which occur in clumps.

From the histological preparations there was no evidence that the microbranchiospines serve any mechanoreceptor function. Mechanoreceptors are commonly found both in the gill rakers and gill filaments of fish (Sutterlin & Saunders, 1969). Nilsson (1984) reports two types of mechanoreceptor, proprioreceptors and nociceptors, in the branchial regions of fish. Although there is a degree of overlap between the two, in general the former are involved in the control of respiration by responding to changes in water pressure in the inhalent current whilst the latter are able to detect slight changes in mechanical pressure caused by suspended particulate material, thus initiating a cough or expulsion reflex.

Considering the above information it is suggested that the microbranchiospines play only an incidental role in filter feeding and protection of the gill filaments by virtue of their situation on the gill arch.

Gill rakers were observed as small projections of taste-buds on the gill arches of fish 9 mm S.L., gill raker number reaching a constant value at 50-60 mm S.L. Such aspects of gill raker development in planktivorous fishes have been described by various authors, Dz. de Ciechomski (1967), Shen (1969), Iwata (1976), King & MacLeod (1976), O'Connell (1981) and Rosen & Hales (1981), (See Lazzaro, 1987, for review).

Most studies on the filter feeding mechanism in fish have assumed a method of mechanical sieving by the gill rakers. Measurement of gill raker length and inter-raker distance in determining mesh size has been performed by King & MacLeod (1976), Durbin (1979), Wright *et al.* (1983). However, King & MacLeod (1976) point out that a filtering mesh in Clupeoids, finer than measurements suggest, results from interlocking of the gill rakers and their denticles on the upper and lower arches. The construction of a fine mesh due to denticles on the gill rakers has been noted by Dz. de Ciechomski (1976) and Shen (1969). Iwata (1976) described the fusion of parts of adjacent gill rakers in the phytoplanktivorous silver carp, *Hypophthalmichthys molitrix.*

The short club-like gill rakers of *O. niloticus* are not typical for a planktivorous mode of feeding whereby mechanical sieving is the only means of filtration. The shortness of the gill rakers and their relatively wide spacing would apparently not produce an effective filter. However, observation of fresh specimens revealed that the gill rakers of neighbouring arches do interdigitate. Although results show the gill rakers to be wider than the inter-raker distances, interdigitation probably occurs due to the structures being fleshy protuberances filled with much loose connective tissue. The interdigitation of gill rakers has been observed in the phytoplanktivorous *O. esculentus* (Greenwood, 1953). From histological sections of whole heads and general dissection, it was apparent that the buccal cavity is dorsoventrally flattened. Liem & Osse (1975) state that such a cavity can be more easily enlarged by movements of the floor of the mouth, whereas a mouth with greater depth may benefit from lateral movement of the side walls. The passage of water through the buccal cavity of *O. niloticus*, due mainly to movements of the mouth floor would not require

excessive lateral movement of the gill arches. This, combined with the tightness of interdigitation due to wider gill rakers than inter-raker distance, may serve to limit the extent of lateral movement of the gill arches maintaining the 'sieve'.

In live fish, the gill arches are orientated so that the anterior face and those of the gill rakers are exposed to the water current. The trailing keel of the gill rakers abut adjacent gill rakers of neighbouring gill arches and perhaps the neighbouring arch itself. In this alignment the taste-buds of the gill rakers face into the water current, whereas the large mucus cells of the trailing keel face into the pores of the sieve. Iwai (1964) gave a comprehensive account of taste-bud occurrence in freshwater, marine and euryhaline species. In fishes with numerous taste-buds in the branchial region, they are more densely arranged along the anterior surface of the gill rakers and arches so that they are near the pathway of engulfed food and respiratory currents. This was observed to be the case in *O. niloticus*. Iwai states that if the gill rakers play an essential part in a filter feeding mechanism, a sensory mechanism for concentrating materials on the gill rakers is expected. The association of taste-buds with the gill arches and rakers of *O. niloticus* from early development (9 mm S.L.) suggests the importance of these structures in filter feeding.

Reutter *et al.* (1974) described three types of taste-buds in the head gut of *Xiphophorus hekleri* Heckel. Due to the depression around the base of some taste-buds, it was hypothesized that they may also serve a mechanoreceptor function. The rows of taste-buds on the anterior edges of the gill rakers may also serve such a function due to their isolation in a single projecting row. The taste-buds of the gill rakers of *O. niloticus* were clearly larger and more numerous than those occurring on the vela, tongue and palate and this is the opposite of what has been found in *X. helleri*. These differences may be related to feeding habit, as *X. helleri* is an omnivorous particulate feeder and, therefore, presumably oral and buccal handling of food items is more important than processing by the gill rakers.

A detailed account of the structure and distribution of taste-buds in the oro-pharyngeal wall of *Cyprinus carpio* is given by Sibbing & Uribe (1985).

Mucus cells were not common in the thick, stratified, squamous epithelium of the gill rakers and arches that contained the taste-buds. However, Reutter (1980) suggests that in fish the large receptor villi of the taste-bud chemosensory cells possess only a thin mucous surface coat, if any, and that they seem to contact taste molecules directly. The perception of certain amino acids as chemical feeding stimulants has been shown for herbivorous fish *T. zillii* by Johnson & Adams (1986).

The anterior face and sides of the gill arch contain small, goblet mucus cells, whereas the keel of the gill rakers is characterized by large, clavate mucus cells. Two distinct types of mucus cell have been shown by Asakawa (1970), Yamada & Yokote (1975) and Bullock *et al.* (1976), although their morphological differences were not associated with differences in histochemical nature. Saxena & Kulshrestha (1981) found two well differentiated mucus cells that secrete different mucosubstances in the epidermis of *Mystus (mystus) vittalus*.

The elongate mucus cells associated with the trailing keel of the gill rakers secrete an acidic mucosubstance. In mammalian systems, viscosity increases as the acidic glycoprotein content of the mucus increases (Jones *et al.*, 1973; Iravani & Melville, 1974), and as the composition of mucus from fish and mammals is similar, its viscosity probably changes in a similar manner (Solanki & Benjamin, 1982). Due to the occurrence and type of mucus produced by the clavate mucus cells, it is proposed that this mucus forms a 'net' in the pores of the branchial sieve. This mucus may also have increased particle capturing properties due to its charged acidic groups. The function of charge in the capture of particles by the Pacific brittle star has been shown by La Barbera (1978).

Flood (1981) found a net-like ultrastructure for the feeding mucus of various marine animals and stated that it could be a fundamental property of mucus. This property would

enhance the ability of mucus secreted by the gill rakers to act as an actual sieve.

The presence of the neutral and neutral/acidic mucus on the anterior face and medial and lateral sides of the gill arch may act in regulating transport of the captured particles to the posterior region of the buccopharyngeal chamber. It is assumed that this mucus would be less viscous.

The only differential distribution of mucus cells on the gill arches of *O. niloticus* was the association of the large clavate cells with the trailing keel of the gill rakers. There were no apparent differences between sections of the same gill arch or between different gill arches. Friedland (1985) showed that there were higher proportions of acidic cells on gill arch IV in the Atlantic menhaden, and suggests that, together with the spacing of branchiospinules on the gill rakers, the gill arches become progressively better at filtering small particles from exterior to interior. Furthermore, it was shown that there was a differential distribution of mucus cells on the gill rakers. Mallat (1981) found that in the pharynx of the larval lamprey alcian blue non-reactive cells were located lateral to alcian reactive cells, in a situation where only small, easily trapped particles were likely to reach.

Sibbing & Uribe (1985) found that mucus cells producing sialomucines occurred in the anterior part of the oro-pharynx of the carp and that sulfomucines only appeared in the posterior part of the pharynx. These mucosubstances were produced by morphologically different types of mucus cells. It was hypothesized that the highly viscous sulfomucines aid in trapping small particles, aggregating them into boluses, and lubrication of the chewing pad, and that the less viscous sialomucines maintain a laminar flow during suction and lubrication of particle handling in the pharynx.

The use of mucus in the feeding mechanisms of anuran larvae has been reported by many workers including Savage (1952), Kenny (1969 a, b) and Wassersug (1972). Kenny (1969b) found the mucus-secreting epithelium of various species to be PAS positive but

alcian blue non-reactive, and stated that it contained no protein, being a glycolipid. However, its viscosity could still be high as Law (1960) states that the 'cord factor' of tuberculosis bacilli, associated with the characteristic tendency of these organisms to aggregate in long cords, is a glycolipid. Lewis (1970) suggests that the proportion of phospholipids may determine the relative viscosity of fish mucus.

A survey of teeth of *Oreochromis mossambicus* (Peters) has been undertaken by Lanzing & Higginbotham (1976). Trewavas (1983) describes the pharyngeal jaws of many tilapiine species and Greenwood (1981) of many *Haplochromis* species.

The taste-buds on the pharyngeal jaws are similar to those of the toothed, masticating apparatus of *X. helleri* described by Reutter *et al.* (1974). The abundance in larvae of 14-20 mm S.L., and decrease as the standard length increases, appears to be correlated with the change in diet from particulate to filter feeding (Trewavas, 1983) and the consequent change in function of the pharyngeal pads from handling single prey items to mucus-bound phytoplankton. Selection of food items may take place at the pharyngeal pads during particulate feeding on single prey items.

A distinctive feature of the mucus cells of the lips and pharyngeal pads was the production of a sulphated mucosubstance. A secretion of sulphated mucus would act as a viscous lubricant and the attachment of this lubricant to the pads would be enhanced by the microridges of the surface epithelium. The increase in mucus cell number observed in larger fish may indicate an increased need for lubrication as the diet changes to phytoplanktivory and the pads function as mechanical rakers of mucus and trapped algae into the oesphagus.

The disappearance of the brown coloured caps of the teeth following decalcification suggests the presence of enameloid. Schmidt (1969) notes that the colour is due to iron oxide associated with the enameloid. Motta (1986) suggests that this iron layer represents a

specialization to harden the teeth, resisting abrasion and cracking. The presence of a hard layer over the pharyngeal jaw teeth of *O. niloticus* would help prevent tooth damage during the raking action of the pharyngeal jaws. Also, the attachment of the teeth to the irregular bone plate by fibrous material would allow slight movement during the raking action of the pads, therefore, making them less susceptible to fracture.

Chapter 3

Quantitative study of filter feeding

3.1 Introduction

The occurrence and growth of blue-green algae in ponds and lakes is well documented, as is their utilization by many fish species (see Colman & Edwards, 1987, for review). Various workers have reported the feeding of *O. niloticus* on blue-green algae (Fryer & Iles, 1972; Moriarty & Moriarty,1973; Trewavas, 1983; Getachew, 1987).

Blue-green algae are unicellular, colonial and filamentous algae with a wide distribution in marine and freshwaters. They also occur terrestrially. The cells do not have a true nucleus and there are no true chromatophores, the pigments being distributed throughout the entire peripheral portion of the cytoplasm. Some of the characteristics possessed by blue-green algae are so similar to those of the bacteria that one older system of classification grouped them under schizophyta (fission plants). In the present classification of the algae the group is given the rank of a Division of the plant kingdom under the name Cyanophyta, a term introduced by Smith in 1938. A discussion of the classification of the blue-green algae is given in Fogg *et al.* (1973).

The species used in this investigation were *Anabaena cylindrica*, a filamentous algae and *Microcystis aeruginosa*, a small, two celled form. The genus *Anabaena* is a member of the family Nostocaceae, Order Nostocales. *Microcystis* belongs to the family Chroococcaceae, Order Chroococcales. Both *Anabaena* and *Microcystis* often occur in water blooms and play a role in the many disturbances resulting from unbalanced conditions in lakes and reservoirs (Prescott, 1969).

Quantitative studies of herbivorous filter-feeders have been undertaken by numerous workers on many species, notably on zooplankton (e.g. McMahon & Rigler, 1963; Frost, 1972; Crowley, 1973; Frost, 1975; Reeve & Walter, 1977; Conover & Mayzaud, 1984) and anuran tadpoles (Seale & Wassersug, 1979; Seale & Beckvar, 1980; Seale *et al.*, 1982). Single studies have also been carried out on various fish species, for instance, on *O. niloticus* and *Haplochromis nigripinnis* (Moriarty & Moriarty, 1973); the Atlantic menhaden (Durbin & Durbin, 1975; Friedland, 1984) and the blue tilapia, *O. aureus* (McDonald, 1985).

Many of the techniques and assumptions used in the quantiative study of filter-feeding were established during early zooplankton studies. In fact, the mathematical basis for most recent studies have been derived from Frost (1972). However, since then a number of papers have addressed the problems of measuring the feeding rates of herbivores (Williams, 1982; McClatchie & Lewis, 1986; Marin *et al.*, 1986).

The ingestion rate formula (see section 2.2.5) used in this study was derived from the equation of Conover & Mayzaud (1984), suggested also by Peters (1984) and discussed by Marin *et al* (1986). This equation was chosen because it describes the actual net changes in the experimental feeding chamber (Conover & Mayzaud, 1984).

When constructing ingestion - concentration curves it is possible to express the axes using various dimensions. Frost (1972) used cell number whereas Crowley (1973) used μg dry weight (comparable to biovolume). Geller (1975) working on *Daphnia pulex* maintained that the maximal volume of the gut fixes the upper limit to ingestion rate and, therefore, the axes of the curves are best expressed on a volumetric basis. Lehman (1976), proposing an energy optimization theory, suggests a regulatory mechanism for ingestion related to energy. Seale & Beckvar (1980) state that tadpoles regulate their food intake on the basis of volume or biomass ingested, or some variable correlated with these e.g. energy, rather than particle size.

The results of quantitative filter-feeding studies have been used to create functional response models. Holling (1959, 1965, 1966) gave a comprehensive analysis of the factors influencing rates of resource utilization by predators stating that the Type I curve (rectilinear) was typical of filter-feeders. However, Ivlev (1961) demonstrated a curvilinear model for ingestion versus food concentration for fish. Both types of curve have been fitted to data and indeed Mullin *et al* (1975) found that for zooplankton data the ingestion - concentration relationship could be described with curvilinear or rectilinear models and that in terms of fit they were statistically indistinguishable. It was also stressed that in addition to the rectilinear and Ivlev models a curve of the Michaelis - Menten type could be justifiably fitted to the data. Such models have also been fitted to data on tadpole ingestion dynamics (Seale & Wassersug, 1979; Seale & Beckvar, 1980; Seale *et al.*, 1982). A Michaelis - Menten model was fitted to the ingestion rates of *S. galilaeus* feeding on plastic microspheres (Drenner *et al.*, 1987).

The processes of zooplankton filter-feeding were analysed further by Lam & Frost (1976) and Lehman (1976) who produced detailed mechanistic models. Lehman (1976) pointed out that as long as the various curves (Rectilinear, Ivelev, Michaelis - Menten) were used by investigators only as simplified representations of experimental data, the lack of agreement over the shape of the curve is probably inconsequential. However, the various models differ most strikingly at low cell concentrations (Mullin *et al.*, 1975) and, therefore, the curves should not be incorporated into simulation models of trophic dynamics.

Peters & Downing (1984) produced an empirical analysis of zooplankton suspension feeding, from data in the available literature, arguing that the statistical models describe suspension feeding more precisely than verbal descriptions of trend.

Thus it was decided to carry out a quantitative study of the filter feeding of *O. niloticus*.

Considering the wide distribution of *Anabaena* and *Microcystis* and the reported ingestion of blue-green algae by numerous fish species the above phytoplankton were used in the filter feeding trials. The two size classes of fish chosen were 40 and 85 mm S.L. the small size class was chosen because, as shown by the morphological observations of chapter two, this was the length at which development of the filter feeding apparatus was complete. The ingestion rates at various algal concentrations were measured in an attempt to produce functional response curves.

3.2 Materials and Methods

3.2.1 Algal Culture

The algae used in this investigation were the blue-green *A. cylindrica* and *M. aeruginosa*. These algae occur naturally in the African lakes, where tilapia are endemic, and are consumed extensively by microphagous tilapia species such as *O. niloticus* (Fryer and Iles, 1972; Moriarty, 1973; Trewavas 1983). Stock cultures were obtained from the Department of Biology, University of Dundee.

The algae were grown as axenic mass cultures in BGII media (Table 2). This medium was devised by Allen (1968) as a modification of GII media of Hughes *et al.* (1958).

Stock cultures were maintained in 100ml pyrex conical flasks, grow-on cultures in 2L pyrex conical flasks and harvest cultures in 6L pyrex carboys. Grow-on cultures were started by adding approximately 20ml of stock culture to 1L of media and harvest cultures started by the addition of approxiamtely 100ml of grow-on culture to 3L of media. Sterile techniques were applied throughout the culture cycle with the air-supply being passed through glass wool filters. Algae were maintained in suspension by aeration and Stuart SM4 magnetic stirrers. Lighting was supplied by 4 horizontally mounted fluorescent tubes. Two fans located at the base of the system produced an air-current from the bottom to the top of the culture apparatus thereby maintaining an operating temperature of

approximately 24 °C.

3.2.2 Experimental System

The experimental facility consisted of a recirculation system used for acclimation of the fish and an inbuilt static system where the filter-feeding trials were performed. Twelve tanks in two rows of six were supplied with recirculating water at a temperature of 26 ± 1°c. Each tank contained a single static 1L feeding chamber and the temperature of the feeding chamber was maintained at 26 ± 1°c by the recirculating water of the acclimation tank (see Figure 5). Aeration of the static-system feeding chambers was acheived via an individual air supply to each chamber. The air supply to each chamber was regulated using plastic screw clamps on the air hoses. Each feeding chamber had a meshed lid, the mesh was squared of sides measuring 2.0mm. Lighting for the experimental system was supplied by an overhead fluorescent tube. The aquarium system was surrounded by black cloth to protect the fish from external visual distractions.

3.2.3 Experimental Protocol

In all trials *O. niloticus* were used at a stocking density of 18.74±2.80 grams Litre $^{-1}$ (n=62) and two size classes, 40 and 85mm S.L. This stocking density approximates the mean fish biomass during the growth period in cages held in fertilized ponds (Coche, 1982).

The filter-feeding trials were single end-point experiments, that is, the algal density in the feeding chambers was recorded at time zero and then again at the end of a defined feeding period, ranging from 40 minutes to 2.5 hours. Three or four replicates and one control chamber were used for each algal concentration. All filter feeding trials were started at 12 noon.

Twenty-four hours prior to the feeding trial the experimental fish were selected from a large stock in the holding tanks and moved to an acclimation tank corresponding to their respective feeding chamber. With the 85mm S.L. fish one fish was used per feeding chamber, however, with the 40mm S.L. fish four fish were used for each chamber. The fish were held for 24 hours in the acclimation tanks during which time they were starved. Two hours before the filter-feeding trial the fish were transferred to their respective feeding chambers.

At the start of the trial the appropriate volume of algal culture to produce a specific algal density was added to the feeding chamber. The density of the algal culture was calculated using a haemocytometer (see below). Five random 10ml samples were taken from each chamber and combined to produce one integrated sample per chamber. The fish were allowed to feed on the algae for a period dependent on the algal density and species of algae. The feeding period was constant for replicates within the treatment. At the end of the feeding period the sample procedure was repeated to produce one final integrated sample per feeding chamber. All samples were preserved with Lugol's iodine. The fish were then anaethetised using benzocaine (Ross & Geddes, 1979) and measured and weighed. The fish from one replicate per treatment were sacrificied and their stomachs dissected out to check for the presence of algae thereby verifying that the fish had been filter-feeding. The volume of water and pH were also recorded for each feeding chamber.

3.2.4 Sample Analysis

Enumeration of both species of algae was achieved using a direct count technique. The principle type of counting chamber employed was an improved Neubauer haemocytometer. However, at low *Anabaena* densities a Sedgewick-Rafter chamber was used. The counting chambers were used in conjunction with an Olympus BH-2 microscope with phase contrast.

The *Microcystis* colonies consisted of two ellipsoid cells joined at their short axes (See Fig. 6). Enumeration of this species proved relatively simple. The colony density was calculated for all concentrations using the haemocytometer. Three counts were made for each integrated sample. One count was made per haemocytometer filling, therefore, three counting chamber fillings were made for each integrated sample. Throughout the period of the experimental trials the *Microcystis* cell size was constant. The biovolume of the colonies was calculated using an ellipsoid shape for the individual *Microcystis* cells. Each colony consisted of two ellipsoid volumes. Measurements were made using an eyepiece graticle. The volume of the *Microcystis* cells was calculated using $V = 4/3\pi abc$; where length = a, height = b and width = c. By observation it was apparent that for *Microcystis* the height and width were equal.

The *Anabaena* colonies consisted of cylindrical cells joined together in filaments. The colony density was calculated using a haemocytometer or Sedgewick Rafter chamber. The biovolume was enumerated using a rather lengthy but nonetheless necessary procedure. The tracings of 50 randomly selected filaments from each sample were made via a drawing tube attached to the Olympus BH-2 microscope. The tracings were then transferred to computer using a digitizer pad coupled to a BBC micro-computer. A software package 'Digit' (Hayes &Fitzke, 1987) enabled statistical manipulation of the stored data. The biovolume of the algae was calculated assuming the filaments had a cylindrical shape ($\pi r^2 h$). The diameter of the *Anabaena* filaments was constant throughout the experimental period. The diameter was measured using an eyepiece graticule.

3.2.5 Determination of Ingestion and Filtration rates

Ingestion rates were calculated in terms of algal biovolume rather than colony number so that the filter-feeding rates of *O. niloticus* on *Microcystis* and *Anabaena* could be compared.

The ingestion rate for each feeding period was computed as :

$$I = \frac{V}{W}\left(\frac{C_o B_o - C_t B_t}{\Delta t}\right)$$

and the filtering rate for each feeding period was computed as:

$$F = \frac{V}{W}\left(\frac{\ln(C_o B_o - C_t B_t)}{\Delta t}\right)$$

where: I ingestion rate ($\mu m^3 g^{-1} h^{-1}$)

F filtration rate ($ml\ g^{-1}\ h^{-1}$)

C_o initial algal concentration in experimental chamber (colonies ml^{-1})

C_t final algal concentration in experimental chamber (colonies ml^{-1})

*B_o mean algal colony biovolume at start of feeding period (μm^3)

*B_t mean algal colony biovolume at end of feeding period (µm3)

Δt experimental duration (h)

V volume of experimental algal suspension (ml)

W wet weight of fish in experimental container (g)

* For *Microcystis* $B_o = B_t = 61.63 \mu m^3$.

No correction was made for algal reproduction in the above equations due to the short experimental periods ≤ 2.5 hours and the fact that there were no significant differences between the initial and final algal biovolume concentrations in any of the controls ($t \leq 2.12$; $n=6$; $P \geq 0.17$).

The above equation for the calculation of ingestion rate is adapted from Conover and Mayzaud (1982) and describes the actual net changes in algal biovolume concentrations in the experimental chamber.

Marin *et al* (1986) explain the erronous use of "mean concentration" in measuring the feeding rates of pelagic herbivores, stating that it produces an artificial increase in the degrees of freedom that may result in the acceptance of nonsignificant regression lines and that it also negates the value of replication. They recommend the use of initial concentration.

This procedure is suited to situations where the monitoring equipment allows very small changes in algal concentration to be detected. Due to the inbuilt error of the counting procedure and, therefore, the percentage decrease in algal concentration required to obtain a significant change during the feeding period (decreases were $\geq 10\%$ in all cases) it was decided to calculate mean algal biovolume concentration during the feeding period for each replicate using the equation:

$$B_c = \frac{(C_t B_t - C_o B_o)}{\ln(C_t B_t / C_o B_o)}$$

where B_c is the mean algal biovolume concentration ($\mu m^3\ ml^{-1}$).

An analysis of variance of mean (Bc) algal biovolume concentrations (Table 3a-d) show that the between-treatment variation was much greater ($F = 169.8 - 4324.6$) than the

within-treatment variation. Therefore, ingestion and filtration rates were plotted versus the mean treatment algal biovolume, $\bar{B}_C$.

3.2.6 SEM study of phytoplankton entrapment

In conjunction with the feeding trials on ingestion and filtration rate, an experiment was carried out to investigate the possible pharyngeal food traps, gill rakers and pharyngeal pads, after exposure to a suspension of algae, *Anabaena*. Two 65mm S.L. *O. niloticus* were left to feed in a suspension of *Anabaena* for one hour afterwhich they were quickly killed and the tissues prepared for SEM as described in chapter two.

Table 2. Composition of BGII algal growth medium (modified from Stanier *et al.* 1971)

Constituent compound	Concentration in stock solution	Inclusion in growth medium
$NaNO_3$	$75.0g. 500ml^{-1}$	$10 ml.L^{-1}$
$MgSO_4 . 7H_2O$	$15.0g. 200ml^{-1}$	$1 ml.L^{-1}$
K_2HPO_4	$15.0g. 200ml^{-1}$	"
$CaCl_2 . 2H_2O$	$15.0g. 200ml^{-1}$	"
Na_2CO_3	$15.0g. 200ml^{-1}$	"
Citric acid	$15.0g. 200ml^{-1}$	"
$FeSO_4 . 7H_2O$	$15.0g. 200ml^{-1}$	"
EDTA	$15.0g. 200ml^{-1}$	"
Trace elements comprising:		
H_3BO_3	$2.680g.L^{-1}$	
$MnCl_2 4H_2O$	$1.810g.L_{1}$	
$Na_2MoO_4 . 2H_2O$	$0.390g.L^{-1}$	
$ZnSO_4 . 7H_2O$	$0.220g.L -1$	
$CuSO_4 . 5H_2O$	$0.079g.L^{-1}$	
$Co(NO)_2 . 6H_2O$	$0.049g.L^{-1}$	

Table 3. One-way ANOVA of mean algal biovolume concentrations in the experimental chambers during the various feeding trials.
(a) *Anabaena*, 40mm S.L. *O. niloticus*
(b) *Anabaena*, 85mm S.L. *O. niloticus*
(c) *Microcystis*, 40mm S.L. *O. niloticus*
(d) *Microcystis*, 85mm S.L. *O. niloticus*

(a)

Source of variation	df	SS	MS	F	P
Treatments	4	$5.249 \cdot 10^{16}$	$1.312 \cdot 10^{16}$	544.34	<0.001
error	10	$2.411 \cdot 10^{14}$	$2.411 \cdot 10^{13}$		
Total	14	$5.274 \cdot 10^{16}$			

(b)

Source of variation	df	SS	MS	F	P
Treatments	3	$5.786 \cdot 10^{16}$	$1.929 \cdot 10^{16}$	169.84	<0.001
error	7	$7.949 \cdot 10^{14}$	$1.136 \cdot 10^{14}$		
Total	10	$5.865 \cdot 10^{16}$			

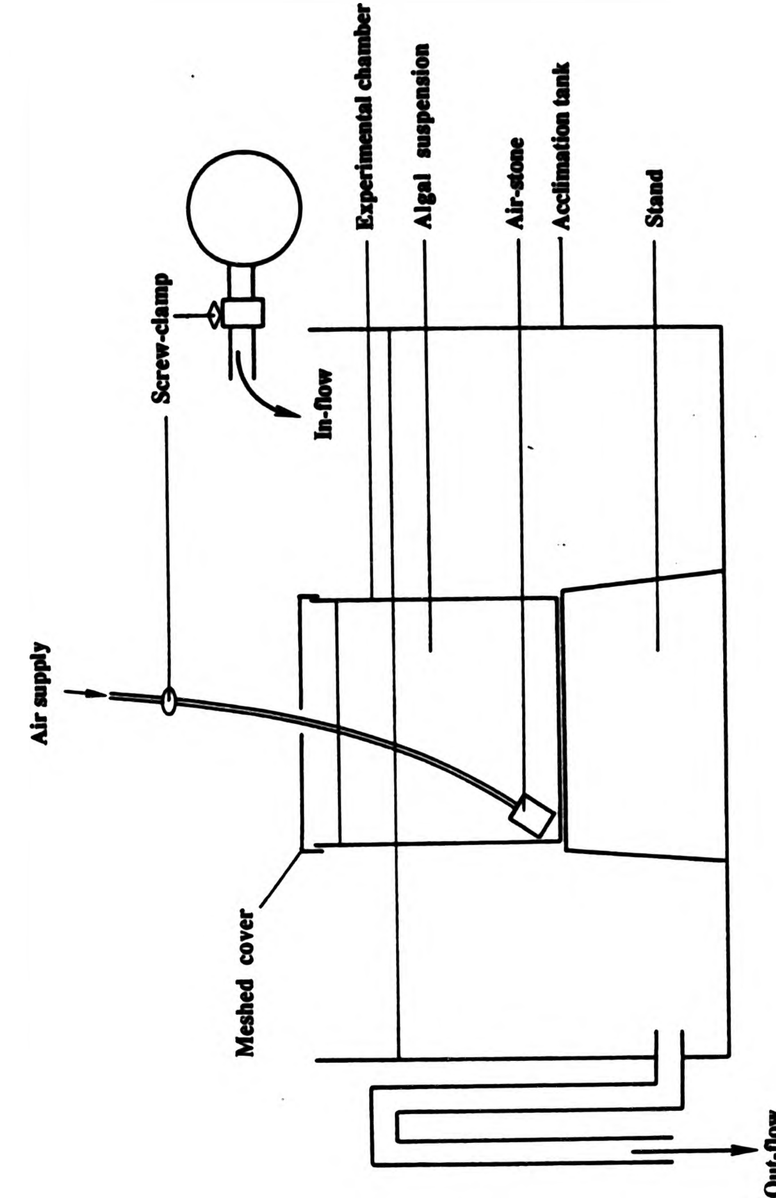
(c)

Source of variation	df	SS	MS	F	P
Treatments	3	$1.307 \cdot 10^{17}$	$4.357 \cdot 10^{16}$	4234.60	<0.001
error	12	$1.235 \cdot 10^{14}$	$1.029 \cdot 10^{13}$		
Total	15	$1.308 \cdot 10^{17}$			

(d)

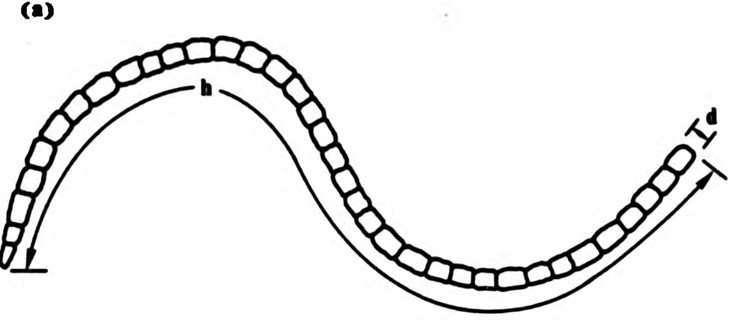
Source of variation	df	SS	MS	F	P
Treatments	4	$2.524 \cdot 10^{17}$	$6.311 \cdot 10^{16}$	1228.31	<0.001
error	15	$7.707 \cdot 10^{14}$	$5.138 \cdot 10^{13}$		
Total	19	$2.532 \cdot 10^{17}$			

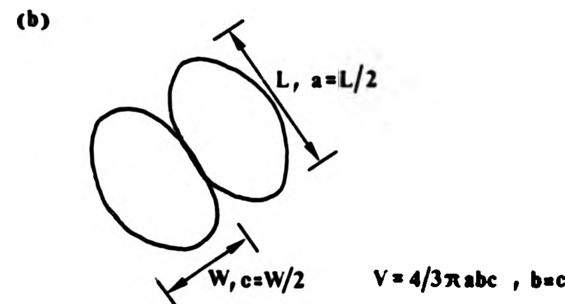
Fig. 5. Diagram of the recirculating aquaria used in the filter feeding trials of *O. niloticus* on blue-green algae.



Air supply
Screw-clamp
In-flow
Experimental chamber
Algal suspension
Air-stone
Acclimation tank
Stand
Meshed cover
Out-flow

Fig. 6. Diagrams of (a) *A. cylindrica* and (b) *M. aeruginosa* showing the dimensions measured for biovolume calculations.


(a)
h
d
V = πr²h , r = d/2


(b)
L, a=L/2
W, c=W/2
V = 4/3πabc , b=c

3.3 Results

3.3.1 Ingestion and Filtration rate experiments

For all feeding trial treatments a student's *t* -test on initial versus final algal biovolume concentrations gave a significant decrease (table 4) showing that grazing of phytoplankton had taken place in the experimental aquaria. This was verified by the presence of algae in the stomach following dissection of sacrificed animals after the feeding period.

With the feeding trial on *Microcystis* the colony biovolume was constant throughout the period of the feeding trials and no size selection during filter feeding could take place. However, although *Anabaena* was harvested at the end of the log growth phase, it was not certain whether the mean colony biovolume was the same in different treatments. Moreover, selection of algae by size may have taken place during the feeding period. Therefore, statistical analyses were carried out on mean colony length between replicates and treatments and within treatments at the start and end of the feeding period.

There were no significant differences between the mean *Anabaena* colony lengths of replicates within a treatment (although due to the great variation in colony length in the cultures the standard deviations were very large) and hence the data were combined for each treatment (n = 150) for the student's *t* -tests on colony length before and after feeding. From tables 5a & b and 6a & b it is apparent that there were significant differences in the mean colony length between certain treatments. Therefore, the expression of ingestion rate in terms of biovolume is more appropriate as it takes into account the differences in the mean biovolume of the algal colonies between the various treatments.

From table 7a & b it is apparent that for each treatment there is a decrease in mean colony length during the feeding period. However, this decrease is only significant in some of the treatments.

For each size class of fish, 40mm and 85mm S.L., and each species of algae, the ingestion, I, and filtration, F, rates were plotted against the treatment mean algal biovolume concentration, $\bar{B}_c$ (Figs. 7a-d, 8, 9a-d, 10a-d). Regression equations were calculated for the ingestion rate curves (table 8). The ingestion rate of 85mm S.L. fish feeding on both species of algae was best fitted using a linear regression over the range of biovolume concentrations studied. The ingestion rate of 40mm S.L. fish gave a curvilinear relationship for both species of algae and was best fitted using a logarithmic regression.

The relationship between filtration rate and algal concentration are shown in figs. 9a-d and 10a-d. The trends for the filtration rate are similar for both size classes of fish within a species of algae but vary between species. The filtration rate with *Microcystis* shows a peak with a sharp decline towards low algal biovolume concentrations and a more gradual decline at high algal concentrations. The peaks in filtration rate occur at similar biovolume concentrations for both the 40mm and 85mm S.L. fish, being 2.455×10^7 and 2.884×10^7 $\mu m\ ml^{-1}$ respectively.

For the 40mm S.L. fish there was a significant difference between the peak filtration rate and that at the low ($t = 7.56$; $n=8$; $P<0.005$) and high algal concentrations ($t = 4.87$; $n=8$; $P<0.05$). There was no significant difference between the filtration rates at the two intermediate concentrations. For the 85mm S.L. fish there was a significant difference between the peak filtration rate and the two low algal concentrations ($t > 2.85$; $n=8$; $P < 0.05$). There was no significant difference between the peak rate and that at the two high algal concentrations.

With *Anabaena* the filtration rate decreases over the range of biovolume concentrations studied. However, with the 40mm S.L. fish the filtration rate appears to reach a plateau at low algal concentrations. This effect is not evident with the 85mm S.L. fish. The plateau for the 40mm S.L. fish occurs at a biovolume concentration of 1.072×10^7 $\mu m^3\ ml^{-1}$. The filtration rate at this concentration is not significantly different from that at the lower

concentration but is ($t > 3.46$; $n=6$; $P<0.05$) from the rates at higher algal biovolume concentrations.

With the 85mm S.L. fish there was a significant difference ($t > 35.48$; $n=5$; $P < 0.001$) between the filtration rate at the lowest algal concentration and those at the higher concentrations.

3.3.2 SEM study of phytoplankton entrapment

From plates 16 and 17a-c it is apparent that the algal filaments are bound in mucus sheets which are trapped by the gill rakers. A mucus sheet caught by the gill rakers of gill arch IV is shown in plate 16, the lower pharyngeal pad is just visible in the background. Plate 17a-c reveals the detail of this mucus sheet at higher magnifications. In plate 17b a number of *Anabaena* colonies can be seen caught in a mucus network that appears to be constructed of long filaments linked together to form a dense sheet. Plate 17c shows these filaments in greater detail and how they are bound in close association with the algal colonies.

Plate 18 reveals the presence of mucus bound *Anabaena* colonies on the lower pharyngeal pad, the inner gill rakers of gill arch IV are visible on the left of the photograph. Plate 19 is a close up of one of the pharyngeal teeth and it can be seen to have a covering of a mucus layer.

Table 4. Students *t* values for initial versus final algal biovolume concentrations of experimental treatments.

Trial		Treatment				
Alga	Fish size (mm S.L.)	1	2	3	4	5
Microcystis	40	4.85**	11.84***	12.06***	4.87**	–
Microcystis	85	9.90***	4.68**	5.30**	4.78**	8.21***
Anabaena	40	23.94***	31.79***	11.53***	6.59**	6.74**
Anabaena	85	4.26**	2.71*	9.46***	5.37**	–

* = $0.05 \geq P > 0.01$ ** = $0.01 \geq P > 0.001$ *** = $P \leq 0.001$

Table 5. Model I ANOVA of *A. cylindrica* colony length in the two size class feeding experiments (a) 40mm S.L. *O. niloticus* (b) 85mm S.L. *O. niloticus*.

(a)

Source of variation	df	SS	MS	F	P
Treatments	4	3437.05	859.26	9.69	<0.002
Error	10	887.05	88.71		
Total	14	4324.10			

(b)

Source of variation	df	SS	MS	F	P
Treatments	3	6800.97	2266.99	22.13	<0.001
Error	7	717.08	102.44		
Total	10	7518.05			

Table 6. Tukey multiple comparison test for *A. cylindrica* colony length in two size class feeding experiments.
(a) 40mm S.L. *O. niloticus*
(b) 85mm S.L. *O. niloticus*

Figures given in comparison tables are 'q' values.
* denotes significance at $P < 0.05$ and ** at $P < 0.005$.

(a)

Treatment number	Treatment Bc ($\mu m^3\ ml^{-1}$)	Treatment mean colony length (μm)
1	$3.0256 . 10^6$	164.23
2	$1.0927 . 10^7$	169.67
3	$2.0086 . 10^7$	126.51
4	$5.9947 . 10^7$	144.06
5	$1.6300 . 10^8$	151.09

Treatment	1	2	3	4
5	2.40	3.40	4.54	1.31
4	3.71	4.71*	3.23	
3	6.93**	7.93**		
2	1.00			

(b)

Treatment number	Treatment Bc ($\mu m^3\ ml^{-1}$)	Treatment mean colony length (μm)
1	$4.4376 . 10^6$	160.65
2	$1.0169 . 10^7$	136.68
3	$3.7487 . 10^7$	132.76
4	$1.7900 . 10^8$	192.63

Treatment	1	2	3
4	4.66	9.58**	9.75**
3	4.07	0.67	
2	3.67		

Table 7. Variance ratio, F and student's *τ* -test for *A. cylindrica* colony length before and after grazing. (a) 40mm S.L. *O. niloticus* (b) 85mm S.L. *O. niloticus*.

* denotes a significant difference in mean colony length after grazing.

$F_{140,140}$ 1.39, $P<0.05$

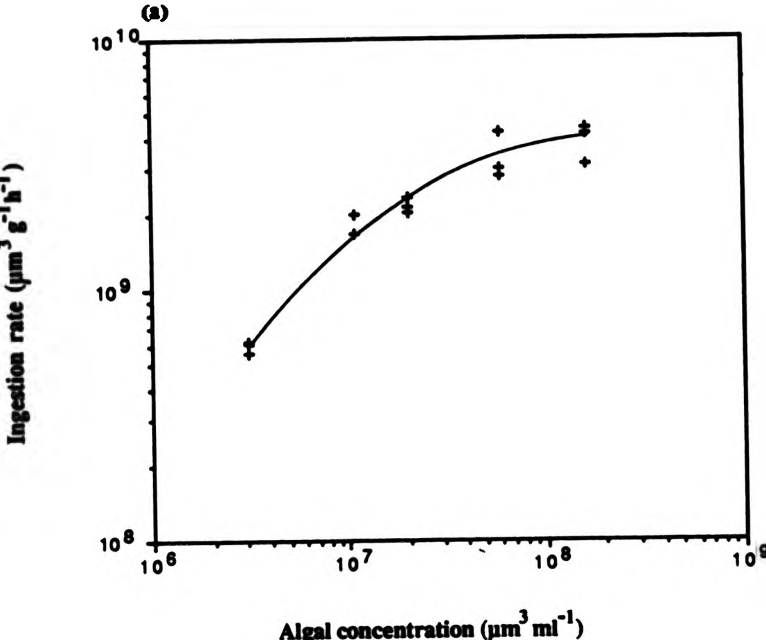
* $F_{90,90}$ 1.52, $P<0.05$

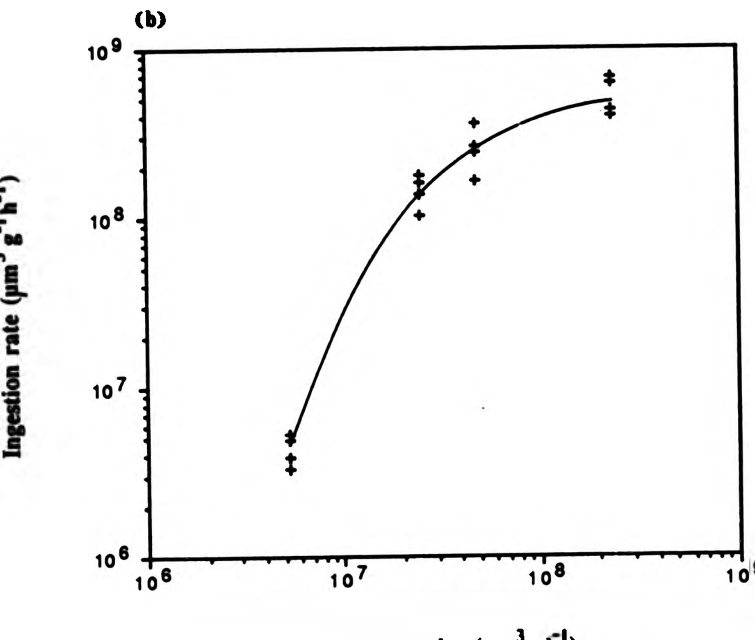
Treatment mean biovolume Bc (μm^3 ml^{-1})	mean colony length ± 1sd (μm) 1. Start (n) 2. End (n)	F	t	P
(a)				
$3.0256 . 10^6$	1. 164.23 ± 96.13 (150)	1.26	2.09	<0.05*
	2. 142.18 ± 85.72 (150)			
$1.0927 . 10^7$	1. 169.67 ± 90.79 (150)	1.08	2.05	<0.05*
	2. 147.73 ± 94.31 (150)			
$2.0086 . 10^7$	1. 126.51 ± 69.26 (150)	1.16	1.17	>0.1
	2. 116.79 ± 74.52 (150)			
$5.9947 . 10^7$	1. 144.06 ± 76.28 (150)	1.06	2.47	<0.02*
	2. 122.60 ± 74.22 (150)			
$1.6300 . 10^8$	1. 151.19 ± 86.50 (150)	1.37	1.85	>0.05
	2. 133.89 ± 73.64 (150)			
(b)				
$4.4376 . 10^6$	1. 160.49 ± 110.59 (99)	+ 1.20	1.63	>0.1
	2. 133.62 ± 121.24 (99)			
$1.0169 . 10^7$	1. 136.82 ± 101.24 (150)	1.28	1.56	>0.1
	2. 119.58 ± 89.48 (150)			
$3.7487 . 10^7$	1. 132.76 ± 83.16 (150)	1.27	2.02	<0.05*
	2. 112.14 ± 93.59 (150)			
$1.7900 . 10^8$	1. 192.63 ± 125.40 (150)	1.06	1.35	>0.1
	2. 173.34 ± 121.65 (150)			

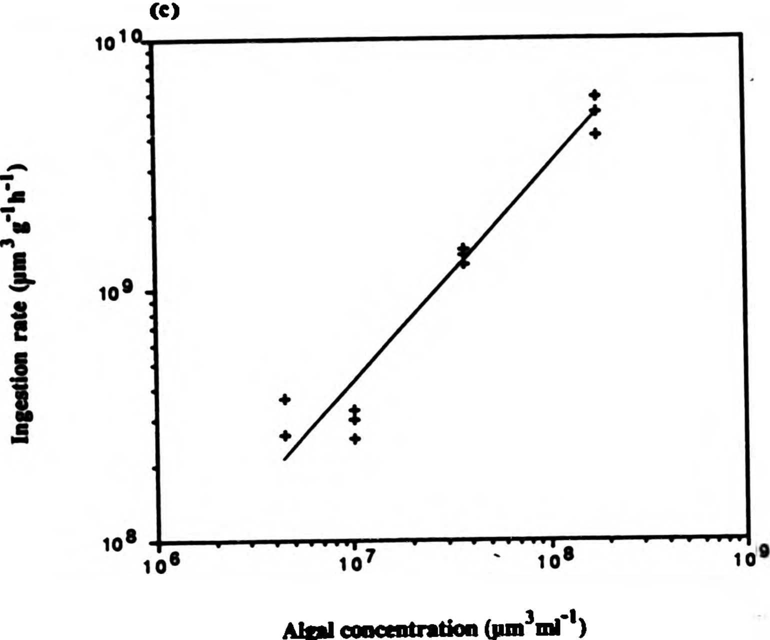
Table 8. *O. niloticus*. Data regression equations of ingestion rate (y, $\mu m^3\ g^{-1}\ h^{-1}$) on mean treatment biovolume concentration (x, $\mu m^3 ml^{-1}$),
$\log_{10} y = a + b \log_{10} X$ for 85 mm S.L. *O. niloticus*
$y = a + b \log_e X$ for 40 mm S.L. *O. niloticus*.

Fish size class (mm)	Algal species	a	b	r^2	P
85	*Anabaena*	2.74	0.84	0.94	<0.001
85	*Microcystis*	0.12	1.09	0.97	<0.001
40	*Anabaena*	$-1.18 \cdot 10^9$	$8.36 \cdot 10^8$	0.89	<0.001
40	*Microcystis*	$-2.21 \cdot 10^9$	$1.41 \cdot 10^8$	0.86	<0.001

Fig. 7. The relationship between ingestion rate, $\underline{I}$ (μm^3 $\underline{g}^{-1}$ h^{-1}) and the mean treatment algal biovolume concentration, $\bar{B}_c$ (μm^3 ml^{-1}) for the two size classes of *O. niloticus* feeding on the two species of blue-green algae.
(a) *Anabaena*, 40 mm S.L. *O. niloticus*.
(b) *Microcystis*, 40 mm S.L. *O. niloticus*.
(c) *Anabaena*, 85 mm S.L. *O. niloticus*.
(d) *Microcystis*, 85 mm S.L. *O. niloticus*.


(a)
Ingestion rate (μm³ g⁻¹ h⁻¹)
Algal concentration (μm³ ml⁻¹)
10¹⁰
10⁹
10⁸
10⁶
10⁷
10⁸
10⁹


(b)
Ingestion rate (μm³ g⁻¹ h⁻¹)
Algal concentration (μm³ ml⁻¹)
10⁹
10⁸
10⁷
10⁶
10⁶
10⁷
10⁸
10⁹


(c)
10^{10}
10^9
10^8
Ingestion rate ($\mu m^3 g^{-1} h^{-1}$)
10^6
10^7
10^8
10^9
Algal concentration ($\mu m^3 ml^{-1}$)

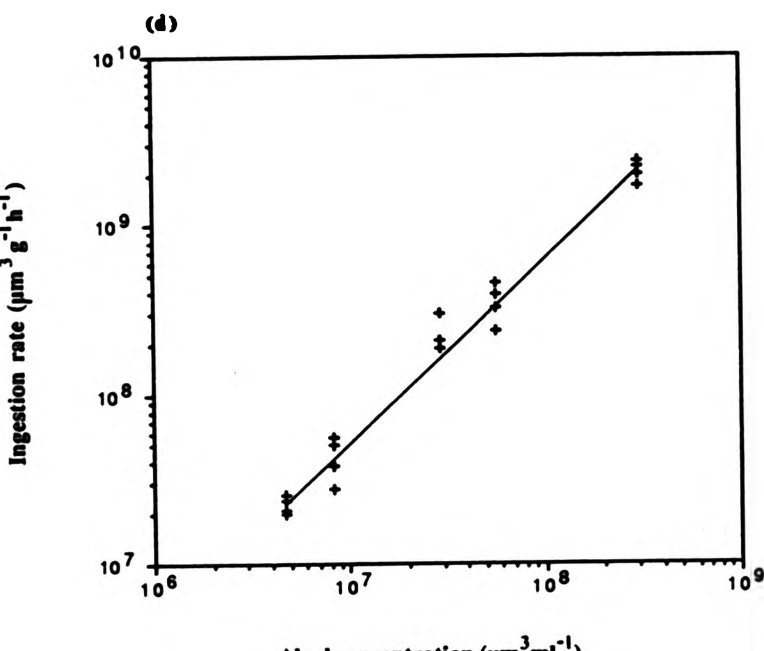

(d)
10^{10}
10^9
10^8
10^7
Ingestion rate ($\mu m^3 g^{-1} h^{-1}$)
10^6
10^7
10^8
10^9
Algal concentration ($\mu m^3 ml^{-1}$)

Fig. 8. Multiple plot of the ingestion rate, I ($\mu m^3\ g^{-1}\ h^{-1}$) versus the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3\ ml^{-1}$).

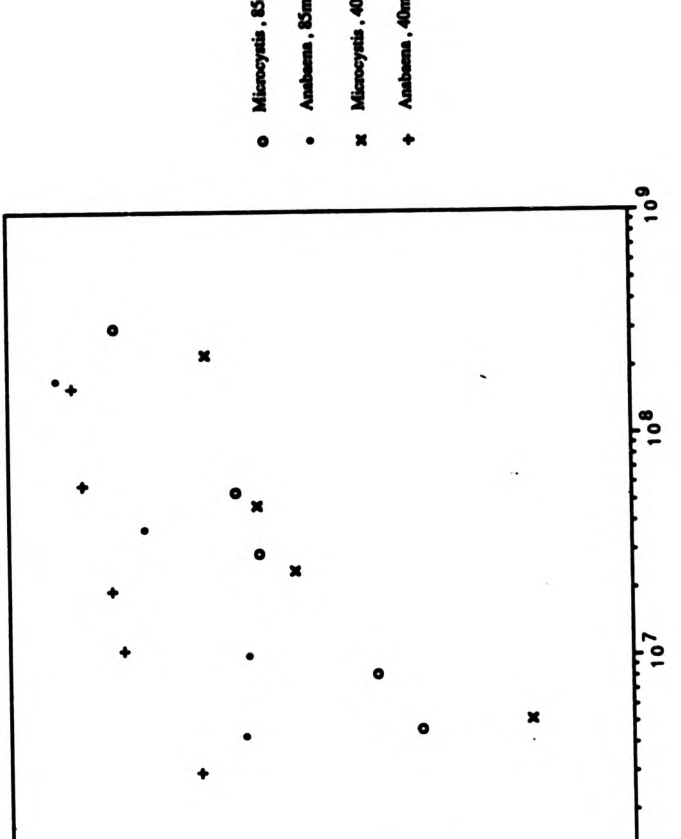
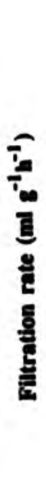
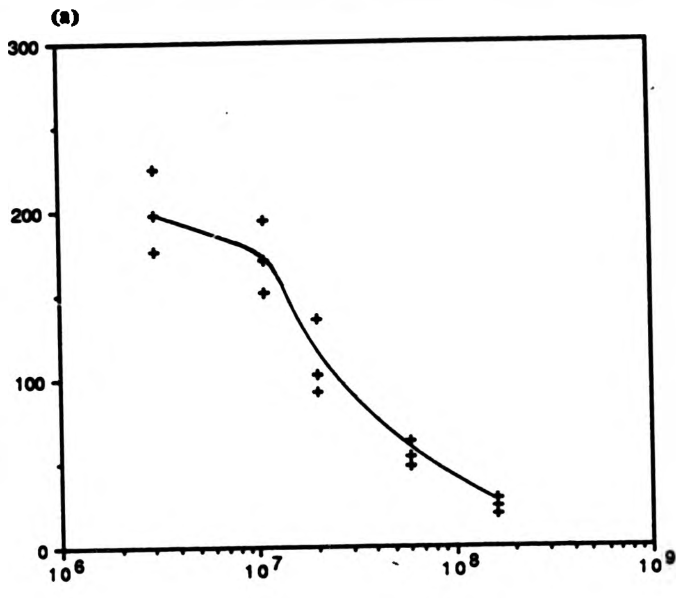
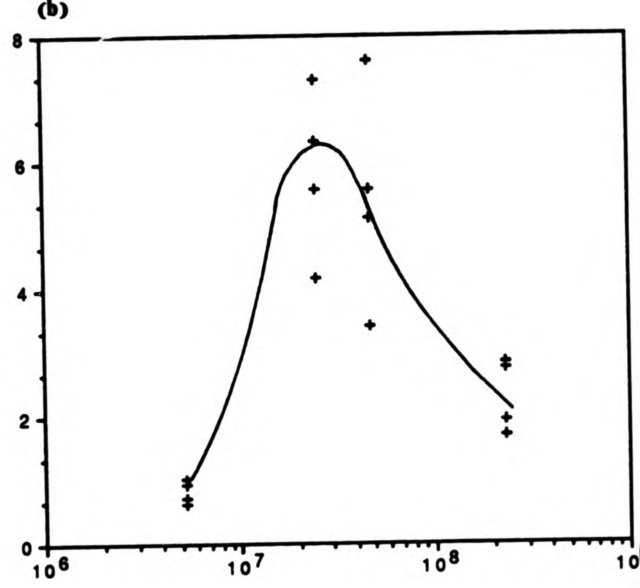

Microcystis , 85mm S.L.
Anabaena , 85mm S.L.
Microcystis , 40mm S.L.
Anabaena , 40mm S.L.
Algal concentration (µm^{3}ml^{-1})
Ingestion rate (µm^3 g^{-1}h^{-1})
10^6
10^7
10^8
10^9
10^{10}

Fig. 9. The relationship between filtration rate, F ($ml\ g^{-1}\ h^{-1}$) and the mean treatment algal biovolume concentration, $\bar{\bar{B}}_c$ ($\mu m^3\ ml^{-1}$) for the two size classes of *O. niloticus* feeding on the two species of blue-green algae.
(a) *Anabaena*, 40 mm S.L. *O. niloticus*.
(b) *Microcystis*, 40 mm S.L. *O. niloticus*.
(c) *Anabaena*, 85 mm S.L. *O. niloticus*.
(d) *Microcystis*, 85 mm S.L. *O. niloticus*.

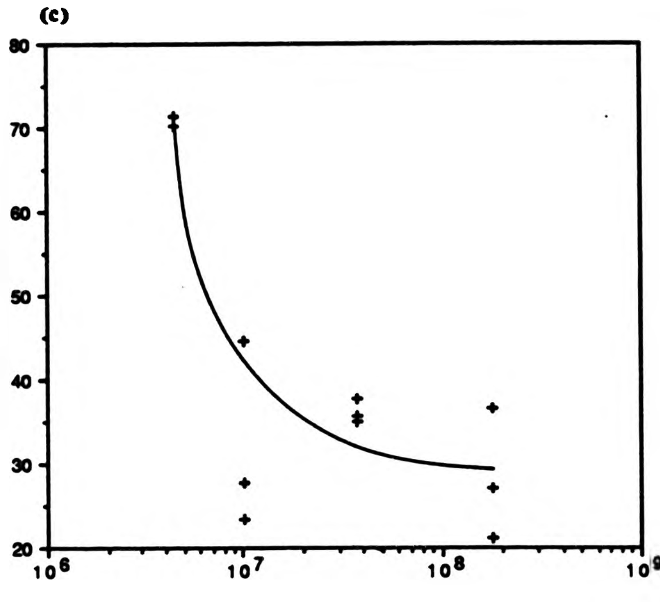




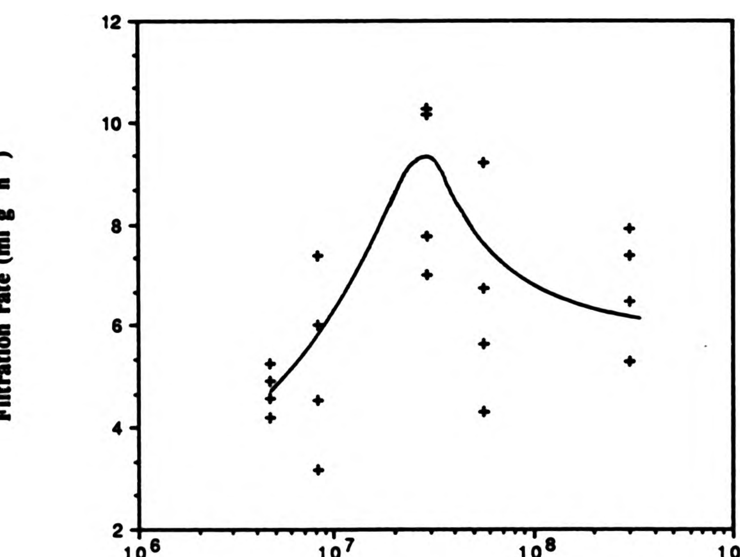
Algal concentration (μm^{3}ml^{-1})



Algal concentration (μm^{3}ml^{-1})



(c)
Filtration rate (ml g^{-1}h^{-1})
80
70
60
50
40
30
20
10^6
10^7
10^8
10^9
Algal concentration (µm^{3}ml^{-1})

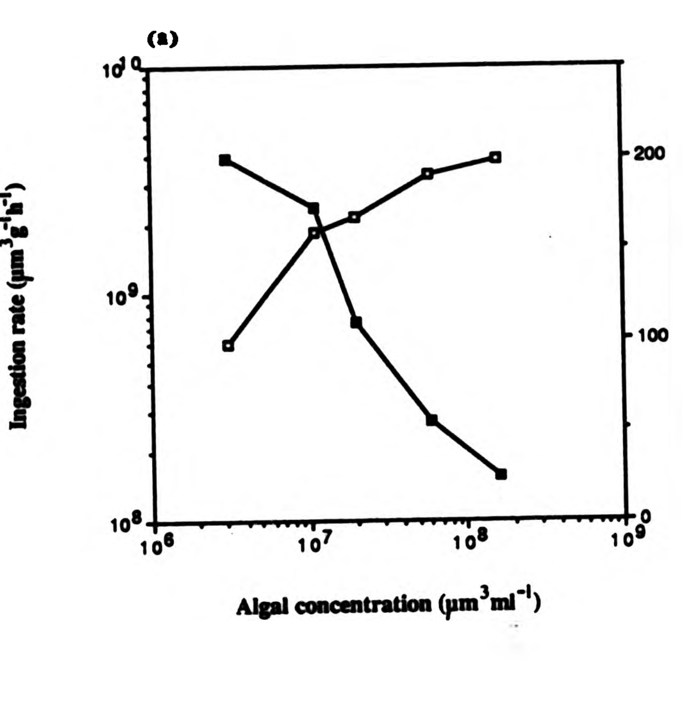


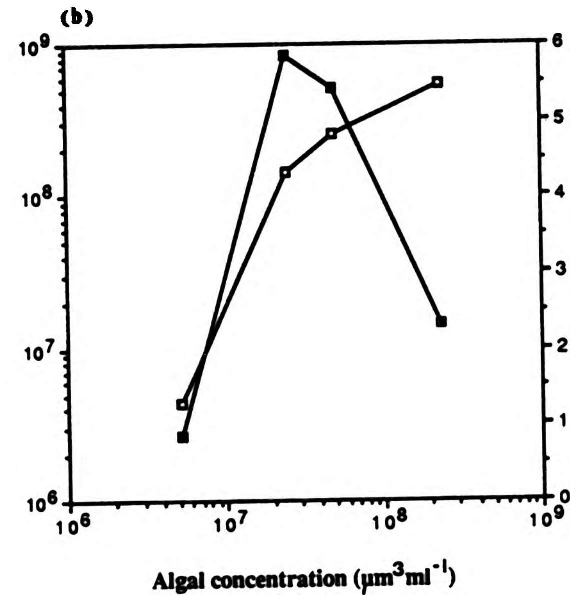
(d)
Filtration rate (ml g^{-1}h^{-1})
12
10
8
6
4
2
10^6
10^7
10^8
10^9
Algal concentration (µm^{3}ml^{-1})

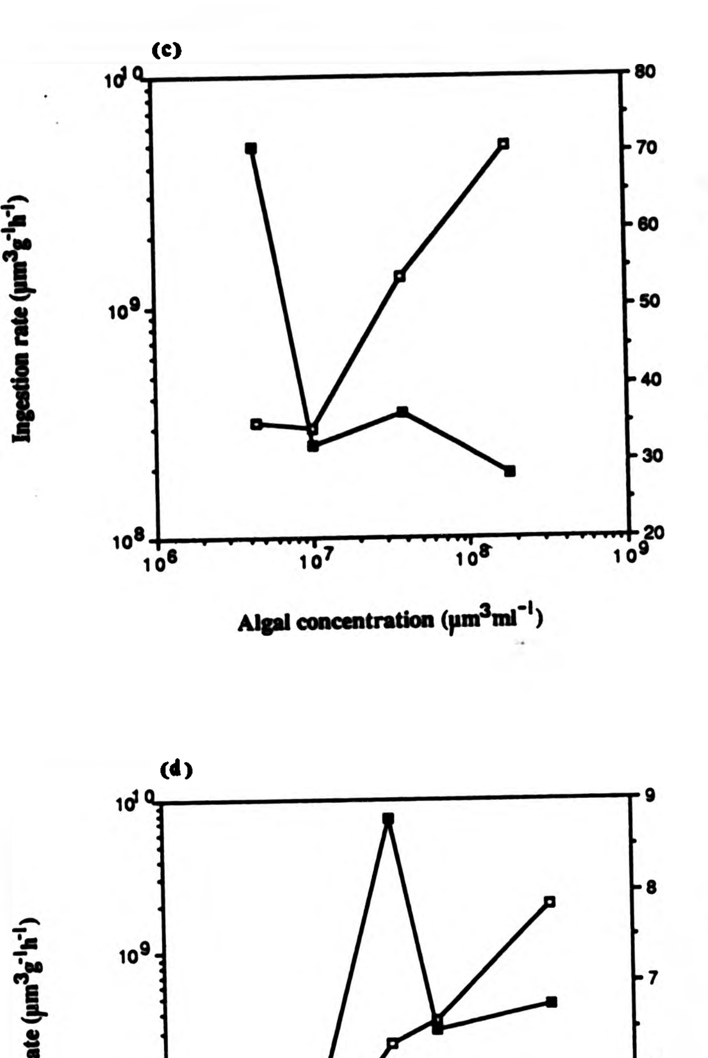
Fig. 10. Combined plots of ingestion rate, I ($\mu m^3\ g^{-1}\ h^{-1}$) and filtration rate, F ($ml\ g^{-1}\ h^{-1}$) versus the mean treatment algal biovolume concentration, $\bar{B}_c$ ($\mu m^3\ ml^{-1}$).

(a) *Anabaena*, 40 mm S.L. *O. niloticus*.
(b) *Microcystis*, 40 mm S.L. *O. niloticus*.
(c) *Anabaena*, 85 mm S.L. *O. niloticus*.
(d) *Microcystis*, 85 mm S.L. *O. niloticus*.

Ingestion rate
Filtration rate


(a)
10^{10}
10^9
10^8
200
100
0
10^6
10^7
10^8
10^9
Ingestion rate ($\mu m^3 g^{-1} h^{-1}$)
Filtration rate ($ml\ g^{-1} h^{-1}$)
Algal concentration ($\mu m^3 ml^{-1}$)


(b)
10^9
10^8
10^7
10^6
6
5
4
3
2
1
0
10^6
10^7
10^8
10^9
Ingestion rate ($\mu m^3 g^{-1} h^{-1}$)
Filtration rate ($ml\ g^{-1} h^{-1}$)
Algal concentration ($\mu m^3 ml^{-1}$)



(d)

Ingestion rate (µm³g⁻¹h⁻¹)

Filtration rate (ml g⁻¹h⁻¹)

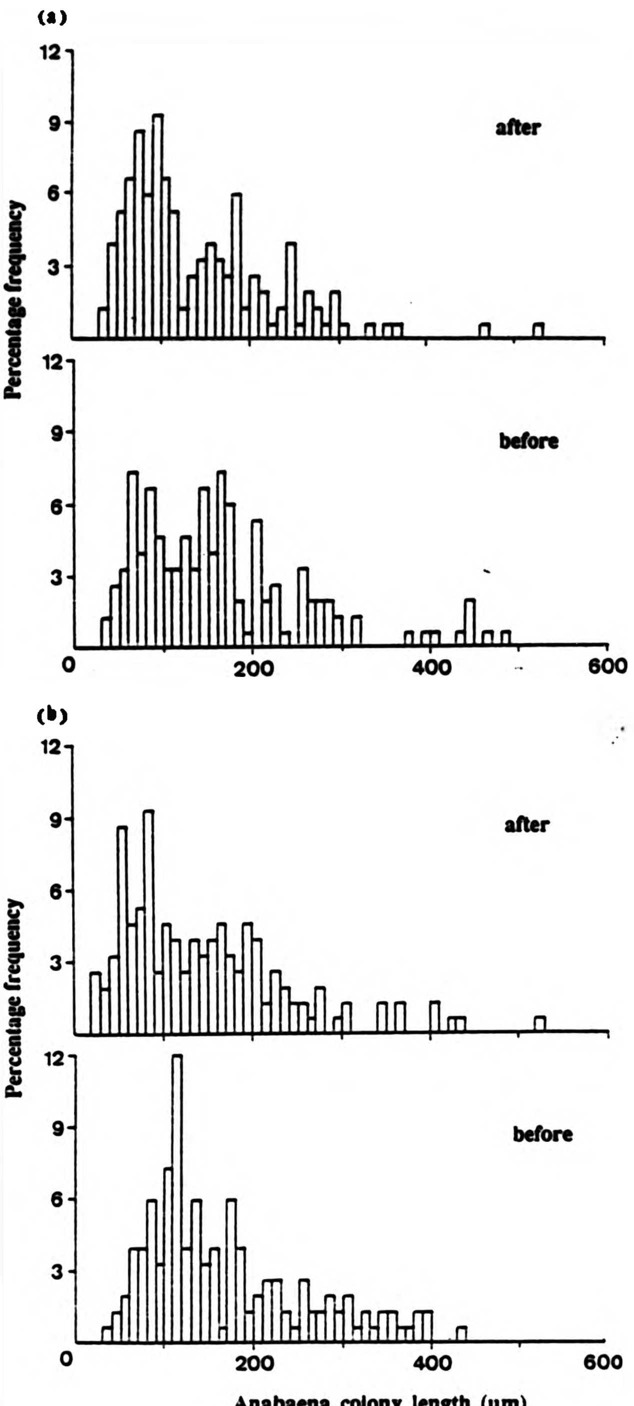
10¹⁰
10⁹
10⁸
10⁷

9
8
7
6
5
4

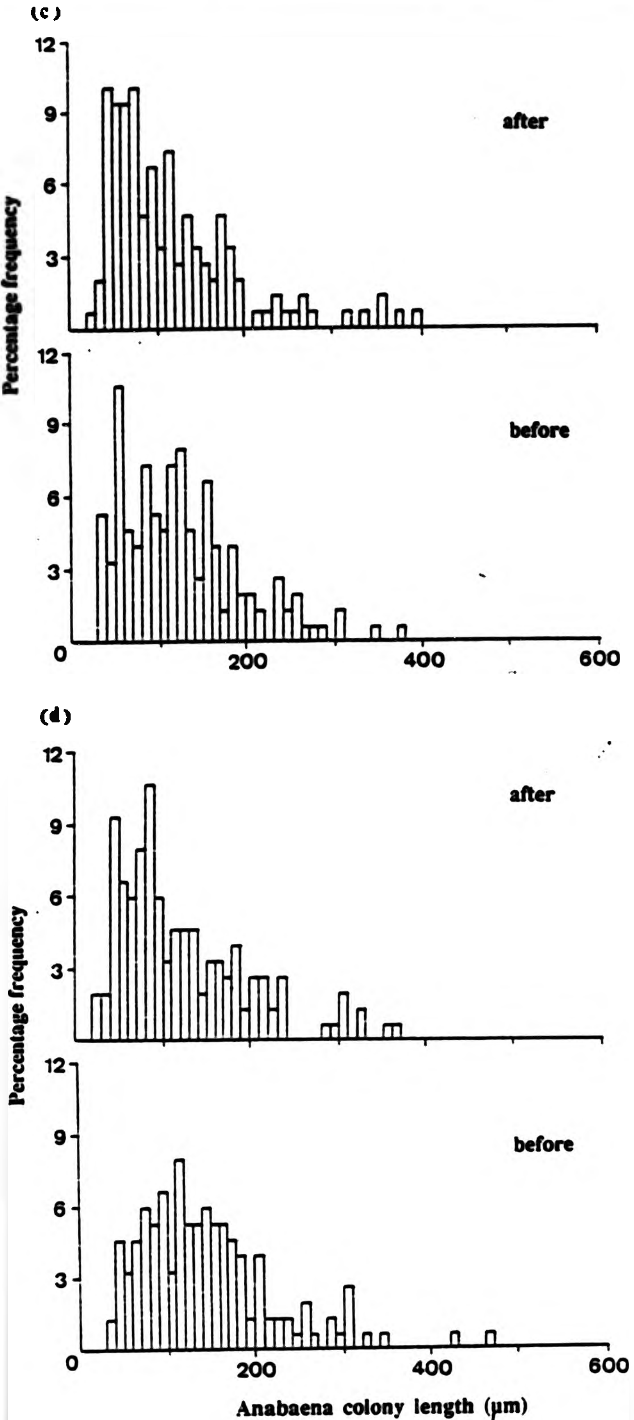
10⁶
10⁷
10⁸
10⁹

Algal concentration (µm³ml⁻¹)

Fig. 11. Percentage frequency histograms for *A. cylindrica* colony length in the experimental feeding chambers before and after grazing by 40 mm S.L. *O. niloticus* for the various mean treatment algal biovolume concentrations.
(a) 3.0256×10^6 μm^3 ml^{-1}
(b) 1.0927×10^7 μm^3 ml^{-1}
(c) 2.0086×10^7 μm^3 ml^{-1}
(d) 5.9947×10^7 μm^3 ml^{-1}
(e) 1.6300×10^8 μm^3 ml^{-1}



(a)
12
9
6
3
after
Percentage frequency
12
9
6
3
before
0
200
400
600
(b)
12
9
6
3
after
Percentage frequency
12
9
6
3
before
0
200
400
600
Anabaena colony length (µm)



(c)
after
before
Percentage frequency
12
9
6
3
0
200
400
600
(d)
after
before
Percentage frequency
Anabaena colony length (µm)

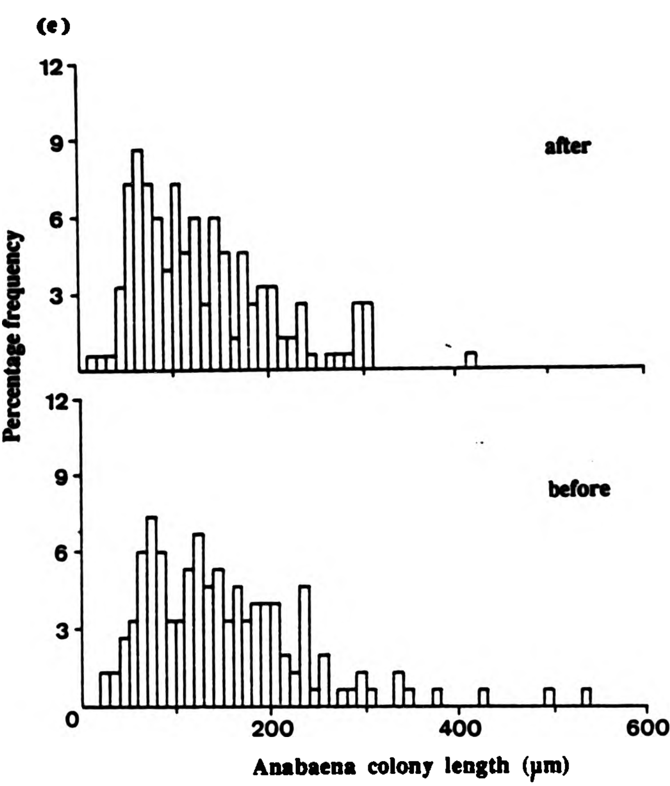

(e)
Percentage frequency
after
before
12
9
6
3
0
200
400
600
Anabaena colony length (μm)

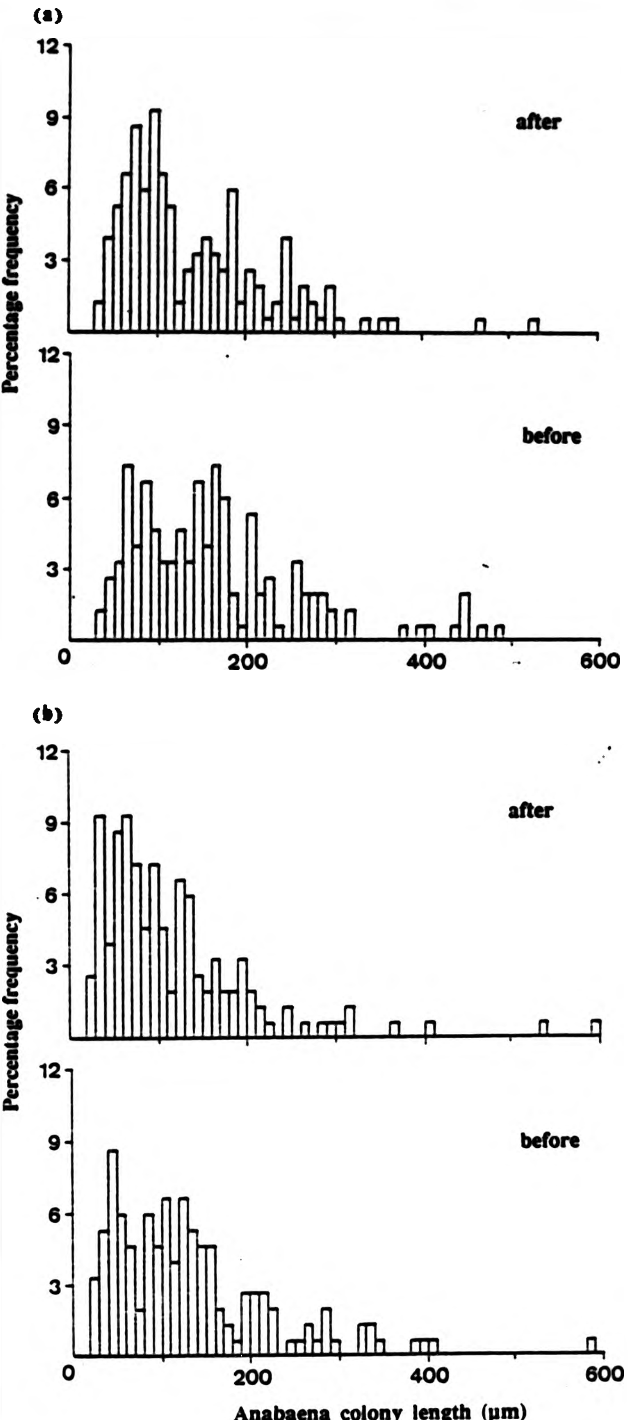
Fig. 12. Percentage frequency histograms for *A. cylindrica* colony length in the experimental feeding chambers before and after grazing by 85 mm S.L. *O. niloticus* for the various mean treatment algal biovolume concentrations.

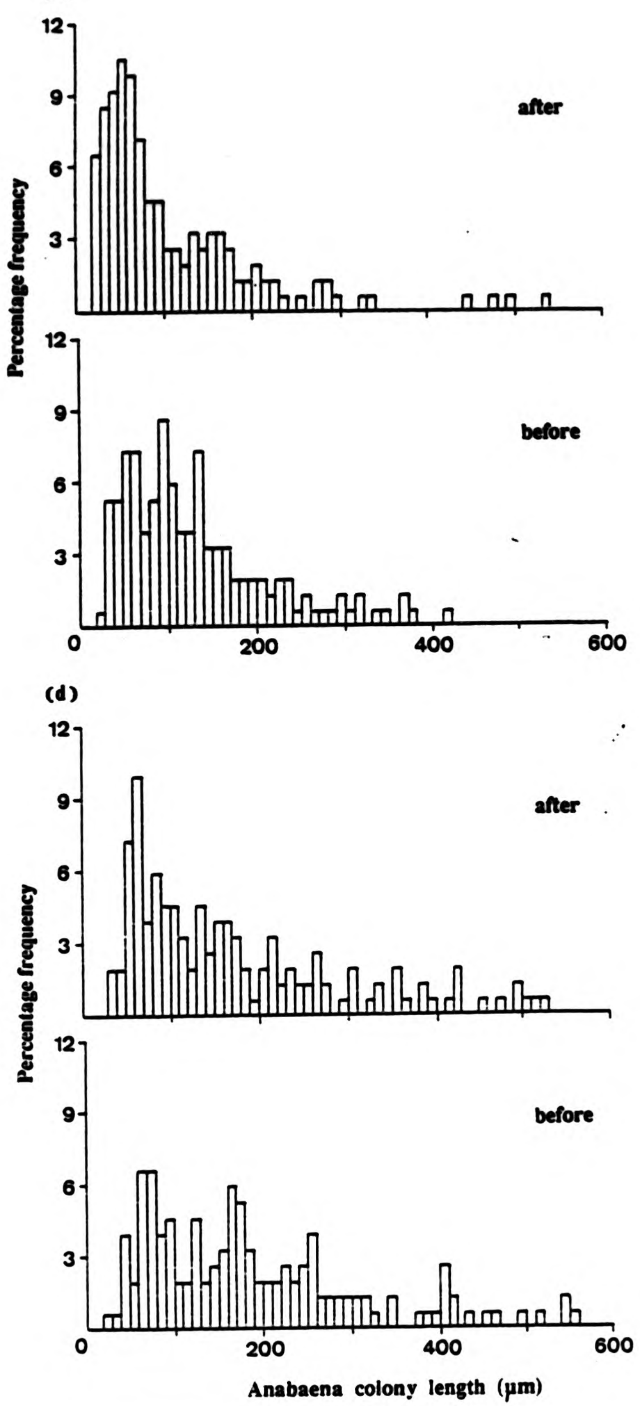
(a) $4.4376 \times 10^{6}\ \mu m^{3}\ ml^{-1}$

(b) $1.0169 \times 10^{7}\ \mu m^{3}\ ml^{-1}$

(c) $3.7487 \times 10^{7}\ \mu m^{3}\ ml^{-1}$

(d) $1.7900 \times 10^{8}\ \mu m^{3}\ ml^{-1}$


(a)
after
before
Percentage frequency
12
9
6
3
0
200
400
600
(b)
after
before
Percentage frequency
Anabaena colony length (µm)



12
9
6
3
Percentage frequency
after
12
9
6
3
before
0
200
400
600
(d)
12
9
6
3
Percentage frequency
after
12
9
6
3
before
0
200
400
600
Anabaena colony length (µm)

Plate 16. SEM of a sheet of mucus entrapped algae caught on the gill rakers of a 65 mm S.L. fish.

Plate 17a-c. Close ups of the mucus entrapped algae.

100 µm

(a)

40µm

(b)

(c)

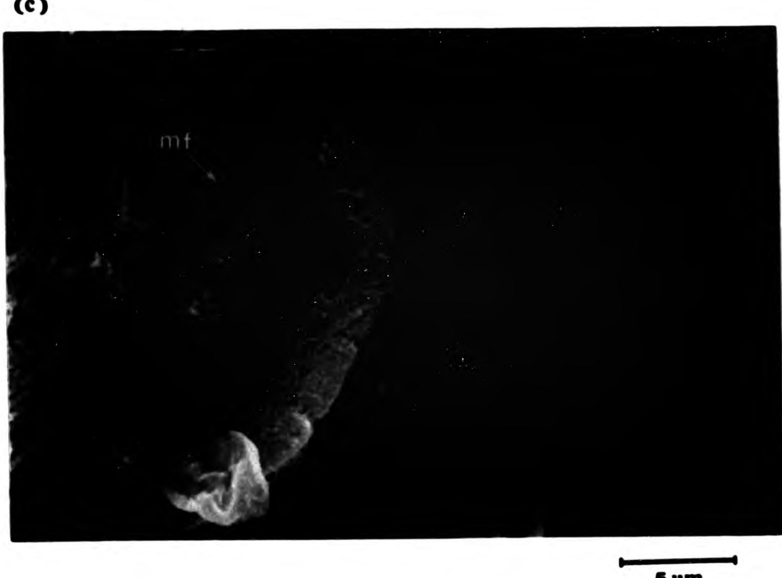
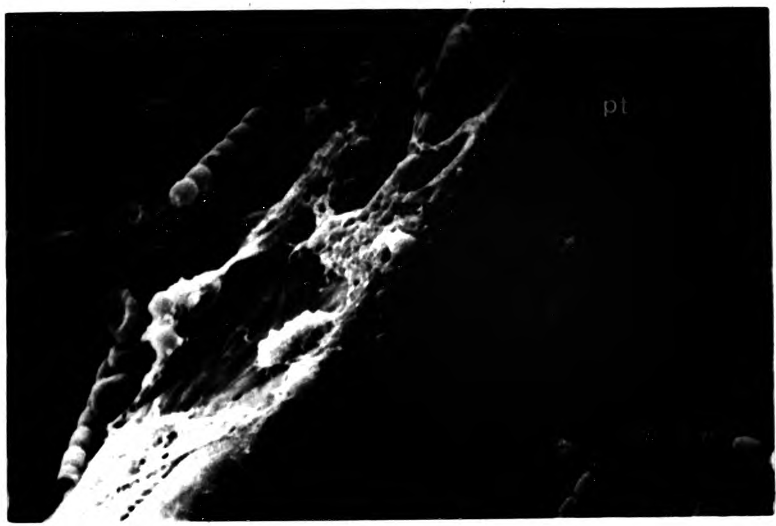


Plate 18. SEM of mucus entrapped algae on the lower pharyngeal pad of a 65 mm S.L. fish. Gill arch IV is visible on the left of the micrograph.

Plate 19. Close up of a pharyngeal tooth and associated mucus covering from the lower pharyngeal pad of a 65 mm S.L. fish.

200 µm


pt
15 µm

3.4 Discussion

Seale *et al* (1982) suggested that the similarity between the responses of invertebrate and tadpole suspension feeders indicates a universality in the fundamental regulatory mechanisms. The results of this survey are similar to previous work on zooplankton (Frost, 1972; Crowley, 1973; Robertson & Frost,1977) and anuran larvae (Seale & Wassersug, 1979; Seale & Beckvar, 1980; Seale *et al.*, 1982).

The asymptotic relationship of ingestion versus concentration for small *O. niloticus* feeding on both *Microcystis* and *Anabaena* is typical for filter feeders. It is consistent with a curvilinear model where filtering surfaces become fully saturated at very high food concentrations as the asymptote is approached, but not with the assumption of saturation at the critical concentration central to the rectilinear model (Rigler, 1961).

The linear relationship for large *O. niloticus* feeding on both species of algae suggests that the biovolume concentration at which saturation of ingestion occurs was not approached. Durbin & Durbin (1975) working on the Atlantic menhaden found that all of their grazing results occurred in the linear phase of the feeding curve.

Although the feeding curves are expressed in terms of biovolume, it was observed that the ingestion rates for *O. niloticus* fed *Anabaena* (Figs. 7a, c & 8) were higher than those fed *Microcystis* (Figs. 7b, d & 8). This suggests that *O. niloticus* can filter *Anabaena* more efficiently than *Microcystis* which would be in agreement with the morphological observations of chapter one. Seale & Wassersug (1979) indicating that tadpoles could capture *Anabaena* more readily than *Chlorella* found that ingestion rates, expressed as biovolume, for both species were similar. This was achieved by adjusting pumping rate, buccal volume displacement, and efficiency of entrapment for the two algae.

The results of this survey show that for each size class of fish the ingestion rates were higher

when fed *Anabaena* with respect to *Microcystis*. This is consistent with the results of Drenner *et al.* (1987) who found that particle ingestion of *S. galilaeus* increased as a function of particle size. Seale & Beckvar (1980), however, found that the maximum ingestion rates of five species of anuran tadpoles were remarkably similar across anuran and algal species. Although maximum ingestion rates were not attained in this survey the results tend to suggest increased feeding on *Anabaena* as opposed to *Microcystis*.

From the results, the maximum ingestion rate for small *O. niloticus* feeding on *Anabaena* would not be much greater than $3.89 \times 10^9 \mu m^3 g^{-1} h^{-1}$. This is comparable with values of 1.14 to $1.25 \times 10^9 \mu m^3 g^{-1} h^{-1}$ for anuran tadpoles feeding on *Anabaena sphaerica* (Seale & Beckver, 1980). The mean wet weights of the five tadpole species ranged from 0.08 to 0.61g and the fish wet weights in this survey from 1.70 to 3.15g. Although the wet weights of the tadpole species differed by a factor of approximately 8 the differences in ingestion rates between species were insignificant. Therefore, the comparison between anuran larvae and fish of this size is useful as the difference in wet weight between the largest tadpole species and the fish is approxiamtely x2 to x5.

In the same paper they also report a maximum ingestion rate of $3.43 \times 10^9 \mu m^3 g^{-1} h^{-1}$ for *Xenopus laevis* tadpoles feeding on *Anabaena*. *Xenopus* tadpoles are obligate midwater suspension feeders with an extensive region of mucus secretory ridges (Seale & Wassersug, 1979). This suggests a more efficient mucus entrapment process for *Xenopus* tadpoles which could explain the increased ingestion rate compared with *Bufo*, *Hyla* and *Rana* tadpoles, having similar oral structures with decreased filtering areas and a smaller buccal volume.

The ingestion rates for the two size classes of fish are similar for the same algal species which is in agreement with Seale & Beckvar (1980). However, the curves would suggest greater maximum ingestion rates for the large *O. niloticus* when compared with the small size class for a particular species of algae. This could be due to increased area of the filter

surface and increased mucus production due to proliferation of the pharyngeal mucus cells (Northcott & Beveridge, 1988).

McDonald (1985) studied the ingestion of *O.aureus* on three species of algae, including *Anabaena flos-aquae*. Although direct values were not given in the paper an ingestion rate for *Anabaena* of 2.81. $10^8 \mu m^3 g^{-1} h^{-1}$ can be calculated for fish of 66 - 83 mm S.L. given a cell size of 6 x 3µm, and an ingestion rate of 8.6 . 10^7 cells h^{-1} for a mean fish wet weight of 13.0g, at a calculated mean algal biovolume concentration of 4.67 . $10^7 \mu m^3 ml^{-1}$. From the linear regression of 85mm S.L. *O. niloticus* feeding on *Anabaena* an ingestion rate of 1.52 x $10^9 \mu m^3 g^{-1} h^{-1}$ is calculated for the above mean biovolume concentration. The lower ingestion rate obtained by McDonald could be due to the fact that the figure was an average obtained over a feeding period of seven hours (as opposed to one hour in this survey), prior to which the fish had been starved for 24 hours. Frost (1972) found the ingestion rate of starved *Calanus* was greater than unstarved animals especially at higher particle concentrations, a trend observed by Wassersug & Hoff (1979) working on tadpoles. It has been stated before that Seale *et al* (1982) suggest a universality in ingestion rate regulatory mechanisms such as satiation or gut filling. Therefore, it is not inconceivable that the fish in McDonald's experiments (1985) had higher ingestion rates initially and that these decreased as the gut filled during the feeding period.

The asymptotic relationship of ingestion versus food concentration for small *O. niloticus* indicates saturation of the filtering mechanism. During the course of the trials, *O. niloticus* were observed expelling boluses of mucus and algae from the operculae opening in a 'cough' like action. Briggs (1985) found both increased opercular beat and cough rates for *O. niloticus* held in water with high algal densities. Such regulatory behaviour would be necessary to maintain a constant ingestion rate at high algal concentrations and has been documented for various species. Seale & Wassersug (1979) observed that at high algal concentrations tadpoles expelled mucus - bound particles from their mouths. McMahon & Rigler (1963) observed a rejection mechanism for *Chlorella* and yeast cells from the food

groove of *Daphnia* at high food concentrations.

The lowest concentration for which ingestion is measurable is called the 'threshold' concentration (Frost, 1975). The ingestion curve of small *O. niloticus* suggest a feeding threshold, whilst the linear ingestion curves for large *O. niloticus* suggest that the lower threshold concentration was not reached. From the nonlinear regressions of small *O. niloticus* the biovolume concentrations at which ingestion would be zero are calculated as $1.35 \cdot 10^6$ and $6.41 \cdot 10^6$ $\mu m^3 ml^{-1}$ for *Anabaena* and *Microcystis* respectively. These concentrations are comparable to those obtained for various species of tadpole feeding on *A. sphaerica*, $3.72 \cdot 10^5$ to $9.73 \cdot 10^6$ $\mu m^3 ml^{-1}$ (Seale & Beckvar, 1980). Seale & Wassersug (1979) found that the threshold concentrations were lower for tadpoles fed large *Anabaena*, an effect predicted by energy optimization models.

The results (Table 7) show a reduction in *Anabaena* colony length during the feeding period. However, the histograms of size frequency (Figs. 11 & 12) give no indication of specific size selection. The results could be due to colony breakage during the feeding process (Deason, 1980). Much literature exists on the size selection of zooplankton. Porter (1977) stated that zooplankton may select against certain classes or species. The size selection of various tilapia species has also been investigated (Drenner *et al.*, 1984b; Drenner *et al.*, 1987), although this was using plastic microspheres, not natural phytoplankton. Seale & Beckvar (1980) found that tadpoles are relatively indiscriminate suspension feeders. It would be expected that a filtering mechanism relying on mucus entrapment would not be too size selective, though Savage (1952) suggested that the efficiency of trapping by a mucus sheet increases as the ratio of surface area / mass of the particles to be trapped increases.

The plots of filtration rate versus algal concentration gave two types of curve for fish feeding on either *Anabaena* or *Microcystis*. This is comparable to the results of Seale & Wassersug (1979) for tadpoles feeding on *Anabaena* and *Chlorella*, respectively. The indirect method for measuring filtering rate assumes 100% retention of particles. Therefore, differences in

entrapment efficiency could have resulted in the differences in filtering dynamics that were observed for the fish feeding on the two algal species.

The filtration curves for *O. niloticus* feeding on *Microcystis* (Fig. 9b, d) are in agreement with a modified curvilinear model with a feeding threshold whereas those for *O. niloticus* feeding on *Anabaena* (Fig. 9a, c) are more comparable to a rectilinear model (Frost, 1975). However, both sets of curves are consistent with sections of the energy optimization model 1 proposed by Lehman (1976), who considered the filter - feeder as an optimal forager.

The SEM study supports the early work of Greenwood (1953) that mucus entrapment of algae is an important mechanism in the filter feeding of tilapias. The appearance of the mucus as a sheet of fibres (Plate 17b, c) is consistent with work by Wassersug (1972) on tadpoles which demonstrated that ingested yeast cells were trapped in a mucus web on the secretory ridges. The mucus covering of the pharyngeal teeth (Plate 19) does not appear to be made up of fibres. The difference in ultrastructure maybe due to the different functions of the mucus. The net-like, fibrous mucus of the gill arches and gill rakers would be useful in particle entrapment, whereas the 'sheet-like' covering of the pharngeal teeth would be advantageous considering a lubricating function (Northcott & Beveridge, 1988). The occurence of intricate, web-like mucus nets is well documented for appendicularian and ascidian filter-feeders (Flood, 1978; Flood & Fiala- Médioni, 1979; Flood, 1981; Flood & Fiala-Médioni, 1981). Humbert *et al* (1984) found a progressive change in the mucus layer along the oesophagus of the eel, *Anguilla anguilla*, from a thick dense layer to a very thin fibrous network. They proposed that the change corresponded to changes in the functions of mucus.

In summary, the quantitative results being comparable to previous work on other aquatic suspension feeders tends to substantiate a universality in the fundamental regulatory mechanism of filter feeding.

The SEM study supports previous morphological observations that mucus entrapment of phytoplankton is important in the filter feeding process of *O. niloticus*. The ability of 40mm S.L. fish to ingest microscopic blue-green algae is consistent with observations that the development of structures associated with filter feeding is complete by this size (Chapter two). This size class is below that at which *O. niloticus* is reported to become phytoplanktivorous in its natural habitat. However, the shift in diet and feeding mode may be due to an ontogenetic migration in the environment to an area with a different food resource.

It was, therefore, decided to carry out an investigation into the feeding ecology and ontogenetic changes in feeding structures of *O. niloticus* populations held in the same environment.

Chapter 4

Thailand Fieldwork

4.1 Introduction

The diverse feeding habits of facultative, planktivorous tilapis in a range of habitats have been studies by many workers. The phytoplanktivorous diet of *O. niloticus* in the wild is well documented (Fryer & Iles, 1972; Moriarty & Moriarty, 1973; Trewavas, 1983; Getachew, 1987). The feeding of *O. niloticus* has also been studied in ponds (Yashouv & Chervinski, 1960, 1961). They showed that, although larger *O. niloticus* could feed on a wide range of prey items available in the pond, the diet changed from omnivorous to phytoplanktivorous as the fish grew and moved into deeper water.

Much work has been carried out on the feeding ecology of *O. mossambicus* in Sri Lankan lakes. Hofer & Schiemer (1983) found the diet of juvenile fish was very uniform, consisting of phytoplankton and mineral sediment. De Silva *et al*. (1984) reported that the feeding habits of *O. mossambicus* were variable from reservoir to reservoir; they ranged from herbivory to total carnivory. The diets of fish in there reservoirs was also shown to vary seasonally, with switches between zoophagy, phytophagy and detritivory (Maitipe & De Silva, 1985). The feeding habits of *O. mossambicus* have also been studied in African water bodies. Bowen (1979) found that both juvenile and adult fish in Lake Sibaya, South Africa fed on benthic detrital aggregates. However, the *mossambicus* population in Hartbeespoort Dam showed an ontogenetic shift in diet, but as they grew they fed increasingly on *M. aeruginosa* and detritus.

Whyte (1975) found that the diet of adult *S. galilaeus multifasciatus* in Lake Bosumtwi,

Ghana was phytoplanktivorous, with the dominant algae being *Microcystis* sp., *Anabaena* sp., *Scendesmus* sp. and *Cosmarium* sp.

The zooplanktivorous feeding and selectivity of *S. galilaeus* has been studied in the laboratory by Drenner *et al.* (1982). Fish less than 20 mm S.L. fed as obligate particulate feeders and were size selective, having highest feeding electivities for large-sized zooplankton species. Fish larger than 62 mm S.L. fed as obligate filter feeders and had positive electives for prey with poor escape ability. However, in Lake Kinneret, Israel the same species was shown to feed primarily on phytoplankton, with *Peridinium cinctum* fa *westii* being the dominant species in the gut contents (Gophen, 1980; Spataru, 1976).

The feeding selectivity of *O. aureus* has been investigated using plankton community impact, mesocosm experiments. Pierce (1983) simply demonstrated algal grazing by this species, whereas Drenner *et al.* (1984b) showed that *O. aureus* suppressed populations of the large-sized algae *Uroglenopsis* sp. and *Ceratium* sp. Vinyard *et al.* (1988) looked at the plankton community impacts of both *S. galilaeus* and *O. aureus*. Both fish suppressed crustaceans and rotifers; however, *S. galilceus* suppressed more and enhanced fewer nanoplankton taxa than did *O. aureus*.

In the natural habitat (where movement between different areas is possible) the feeding mode and diet of *O. niloticus* changes from omnivorous particulate feeding, when young fry, to phytoplanktivorous filter feeding by 60 mm total length (Trewavas, 1983). Investigations of feeding apparatus and diet have been documented for a number of planktivorous fish species. A change in the diet from zooplanktivory to phytoplanktivory of the Argenting anchovy, *Engraulis anchiota*. corresponding to development of the filter apparatus has been demonstrated by Dz. de Ciechomski (1967). Similar findings have been documented for *E. japonica* (Shen,1969); for pilchard, *S. ocellata*, and anchovy, *E. capensis* (King & MacLeod, 1976) and for *E. mordax* (O'Connell, 1981). Rosen &

Hales (1981) correlated the development of the gill rakers with the zooplanktivorous filter feeding of adult paddlefish, *Polyodon spathula*. Similarly, the phytoplanktivorous feeding habit of the silver carp was correlated with its gill raker morphology (Iwata,1976). Datta & Munshi (1984) during an SEM investigation observed that the lamellated gill-rakers of *Rhinommugil corsula* made an efficient sieve for straining phytoplankton as did Hossler *et al.* (1979) for the mullet, *Mugil cephalus*.

The above studies involve a branchial apparatus that is assumed to work as a passive sieve. However, as proposed in Chapter two, the gill raker morphology of *O. niloticus* does not appear to represent an efficient sieve and mucus entrapment is proposed. A number of authors have considered the role of branchial mucus in entrapment of fine food particles. Greenwood (1953) observed the interdigitation of gill rakers in the phytoplanktivorous *O. esculentus* and proposed mucus entrapment of algae in the buccal cavity. Similar mechanisms have been proposed for the estuarine round-herring, *Gilchristella aestuarius*, (White & Bruton, 1983) and the Atlantic menhaden, *B. tyrannus*, (Friedland, 1985). Gibson (1988) observed mucus glands on the gill arches of the herring, *Clupea harengus*, but was not certain whether they contributed to adhesion of particles.

Prolactin is known to control mucus secretion in some teleosts (Johnson, 1973). According to Marshall (1976) gill, but not skin, goblet cells in *Leptocottus* are under endocrine control, and are particulary influenced by prolactin and cortisol. Mattheij & Spranges (1969) demonstrated that when the activity of the prolactin secreting cells is reduced *Anoptichthys jordani* had fewer gill mucous cells than control fish. This was also shown for *Mugil cephalus* and *M. capito* (Blanc - Livini & Abraham, 1970). Wittouk (1975) found that prolactin injection caused a pronounced increase in the number of gill mucus cell in axolotls. These studies suggest that mucus production is correlated with mucus cell number, an observation which was further substantiated by studies on the Sialic acid, N-acetylneuraminic acid (NANA), a component of fish mucus. Olivereau & Lemoine (1972) assumed that the NANA concentration was related to goblet cell number in

the epidermis of the eel, *Anguilla anguilla*. Pickering (1974) demonstrated that the NANA concentration in the epidermis of trout and char was directly proportional to the mucus cell concentration.

Leatherland & Lam (1969) and Marshall (1976) used a 'score system' to estimate mucus cell numbers. However, Solanki & Benjamin (1982) measuring mucus cell concentrations in the gills, buccal cavity and epidermis of the stickleback, *Pungitius pungitius*, pointed out that such quantitative methods may not be sensitive enough for non-parametric statistics, leading other workers to limit investigation to qualitative analyses. Problems with counting the mucus cells in the gill rakers of *O. niloticus* have been reported in an earlier study (Northcott & Beveridge, 1988).

The anatomical studies reported in this work suggest that the filter feeding apparatus of *O. niloticus* is fully developed by approximately 40 mm S.L. Furthermore, the ingestion experiments described previously (Chapter three) demonstrate that fish of this length can ingest *Anabaena* and *Microcystis*. It was decided, therefore, to investigate the possible 'switch-over' period and ontogenetic shift in dietary habit of *O. niloticus* held in the same environment. The study involved an investigation of the feeding ecology and the structure and development of the feeding apparatus in *O. niloticus* held in cages in fertilized ponds.

The feeding ecology was studied by stomach content analysis. The wide diversity in the size and type of food items in the diet of fish in this study required that a combination of objective methods and subjective methods were adopted from the available literature. Review and discussion papers on the methods involved in stomach content analysis are numerous (Hynes, 1950; Pillay, 1952; Langler, 1956; Windell, 1968; Windell & Bowen,1978; Berg, 1979; Hyslop, 1980). The methods chosen were those relevant to the aims of the dietary study, the aims being:

1. Consideration of temporal variation in the diet with changing conditions in the cages.

2. Diet comparison between different size classes of the same species.

3. Investigation of diurnal feeding rhythms.

As the results of Chapters two and three substantiate a theory of mucus entrapment in the feeding mechanism of *O. niloticus* it was decided to investigate the histology of the filtering apparatus. Possible changes in both ontogenetic and diurnal pharyngeal mucus cell histology were investigated to reveal any correlation between mucus production and histochemistry with the feeding ecology of the fish. Mucus cell occurence and morphology were used as an indictor of mucus production, however, only a qualitative analysis was performed.

4.2 Materials and Methods

4.2.1 Experimental Design

The experiments were conducted in a large pond, 100m x 25m, which was organically fertilized to encourage a heavy bloom of plankton (see Colman & Edwards, 1987 for discussion). Ideally the water would have contained minimum organic/detrital material but dense populations of plankton with blue-green algae dominating after a period of species succession. Three cages, 1.8 x 1.8 x 0.9m deep, were located in the pond (see frontispiece) and were stocked with groups of *O. niloticus* of weight classes 2.66 ±1.05 g (small); 6.95 ± 2.15g (medium) and 12.97 ± 3.05g (large), (n=30, from an initial sample weighing). Approximately 4000 of the small size class fish were stocked in cage C, 800 medium sized fish in cage B and 400 large size class fish in cage A. The fieldwork was carried out over a period of 45 days. The nets were changed at 10 day intervals because of the high rate of fouling. No supplementary feed was given, therefore, sample weighings were made so as to calculate growth rates.

4.2.2 Project Investigations

I General Water Quality.

Water quality was measured throughout the duration of the project. The parameters measured were pH, temperature, dissolved oxygen (D.O.), chlorophyll 'a', phaeophytin and secci-disc depth. These parameters were recorded from one to three times daily in each cage and in the pond, in the early morning, at midday and late afternoon. During the diurnal feeding trials the temperature and D.O. were measured every three hours. Temperature and D.O. were measured at the pond using a Clandon YSI model 57 oxygen meter. One litre water samples were brought back to the laboratory for pH and pigment analysis. The pH was recorded using a bench pH meter and chlorophyll 'a' determined according to APHA (1985).

Water samples for plankton analysis were collected using a wide-necked (8cm diameter) 2L bottle fixed to the end of a 2.5m pole. Samples were taken back to the laboratory and allowed to settle in 2L graduated measuring cylinders. Settling was aided by the addition of Lugol's iodine, this also preserved the final settled sample. After settling, the supernatant was carefully siphoned off using a rubber tube and the concentrated sample transferred to 50ml plastic bottles. Samples were preserved in the dark at 4°c.

II Diurnal Trials.

Two feeding trials were carried out from 6.11.87 to 7.11.87 and 9.11.87 to 10.11.87 on fish of ≈ 80mm S.L. For the first trial eight fish were netted every three hours from 0900 to 0600 hours, three for stomach content samples (see below) and five for stomach dry weight analysis (see below). Gill arch tissue was taken at 1200, 1800, 2400 and 0600 hours. For the second trial five fish were taken every three hours as described above. The stomach pH was measured and the stomach and guts including contents were dissected out, separated and placed in individual foil dishes for dry weight analysis (see below).

One feeding trial of fish 35mm S.L. was carried out from 17.11.87 - 18.11.87. The procedure was as for the first trial of 80mm S.L. fish, except stomach dry weight analysis was not performed.

III Ontogeny of Feeding apparatus and Habits.

Fish of size classes 35, 50,65 and 80mm S.L. were studied. Five fish per size class were sampled. Stomachs were preserved for stomach content analysis and feeding apparatus fixed for histological and scanning electron microscope studies.

The final composition of the water column in the pond and cages was a dense *M. aeruginosa* bloom in suspension. During this phase samples of fish of size classes 40 and 80mm S.L. were netted for collection of stomach contents and histological fixation of the feeding apparatus.

4.2.3 Sampling Protocol

I Sampling Technique

Fish were sampled from the cages using a dip net fixed to a 2m bamboo pole enabling fish to be sampled from various parts of the cage. The bamboo walkway next to the cages facilitated such sampling. Immediately the fish were netted from the cages they were anaethetised in a bucket containing benzocaine. The fish were taken back to the laboratory for further investigation. All fish were measured and weighed prior to being killed.

II Stomach Content Collection

The stomach of fish ≥ 60mm S.L. were carefully dissected out and the oesophageal and pyloric openings closed with thread tied in a surgeon's reef knot. These stomachs were then placed in 10% buffered formalin in labelled plastic pots. Fish ≤ 50mm S.L. had

an incision made along the belly, from the anus to the rear of the pharyngeal chamber. The incision penetrated into the abdominal cavity. The whole fish were then placed in 10% buffered formalin in labelled pots.

III Tissue Fixation

Gill arches of large fish or whole heads of small (≤ 40mm S.L.) fish were processed for histology or SEM using the techniques described in sections 2.2.3 and 2.2.4. Tissues for histology were stored at 4°c at the Asian Institute of Technology (A.I.T.), Bangkok. Processing and embedding in wax was carried out at the National Inland Fisheries Institute (N.I.F.I.), Kasetsart University, Bangkok. Tissues for SEM were stored in 70% acetone at 4°c and were transported back to Stirling where final processing took place.

IV Stomach pH

Stomach pH was recorded with the use of a MI-415 Micro-combination pH probe (Microelectrodes Inc., U.S.A.). Fish were sampled from the cage and placed in a bucket containing pond water. They were transported to the laboratory where they were anaethetized with benzocaine and killed. Immediately after being killed the belly wall was carefully cut open to expose the stomach. The stomach was removed and an incision made in the stomach wall. The pH probe was inserted into the stomach contents and the reading displayed via a digital laboratory pH meter which gave values to 2 decimal places. The time scale from fish sampling to completion of pH recording was never greater than 20 minutes. An earlier experiment demonstrated that there was no change in the pH of the stomach contents of a dissected stomach during a 30 minute period.

V Dry weight of stomach contents.

Individual stomachs and contents were placed in separate, preweighed dishes and dried to constant weight at 70°c. The empty stomachs of thirty starved fish of weight range 8.9 - 22.3g were also dried to constant weight. A plot of empty stomach dry weight

(mg) versus fish wet weight (g) was constructed (Fig. 13) and a regression equation calculated, $Y = 0.749X + 1.78$ ($r^2 = 0.72$) where Y is the stomach dry weight and X the fish wet weight. Using this data it was possible to calculate the expected dry weights of empty stomachs and hence the dry weight of the stomach contents only of the fish in the diurnal feeding study.

4.2.4 Sample Analysis

I Water Samples

Phytoplankton were enumerated using a modification of the Lackey drop (microtransect) counting method (APHA, 1985). A 50µl aliquot was placed on a microscope slide using an automatic micro-pipette. A coverslip was gently lowered over the water drop using watchmakers forceps. The phytoplankon were identified to genus level. Algae were enumerated when they occurred in significant numbers, > 400 l^{-1}, otherwise their presence was merely noted. When cells occurred in colonies the colony was counted as the unit. The small green unicells *chlorella* and *chlorococcum* were counted and sized as one group. The phytoplankton were identified and counted using a x40 objective in at least 10 fields of view per slide. Three replicate slides were analysed for each water sample. The exceptions to this method were *Microcystis* and *Phacus* which, due to their increased size, were enumerated according to the method adopted for the zooplankton (see below).

Zooplankton were enumerated and identified using a Sedgewick-Rafter counting chamber. A 1 ml aliquot was transferred to the chamber using a wide bore pipette. The microscope objectives used were x10 and x20. Counts of three replicate chambers were made. Only whole organisms or heads of organisms were counted.

The linear dimensions of both the zooplankton and the phytoplankton were measured using an eyepiece graticule. The biovolumes were calculated using simple geometric figures

approximating the shape of the organism. Figures chosen were collaborated with those in the published literature (Tables 9 and 10). Algal biovolumes were calculated from specimens in an integrated sample of the various individual dates. Zooplankton biovolumes were calculated for separate cages and sampling dates, except *Cladocera* where the biovolume was calculated from combined data.

The percentage volume composition of the various components in the water samples was judged by eye in 5% intervals. The components were detritus, phytoplankton (excluding *Microcystis*), *Microcystis* and zooplankton. Using the actual density and biovolume values for the phytoplankton and zooplankton the percentage volume compositions were also calculated using a numerical method. The two methods were then compared (Table 11).

II Stomach Samples

Each stomach was cut open and the contents diluted in a known amount of water. Algae was identified as before. Enumeration was not attempted as the formation of unicells and colonies into large indistinguishable clumps would have biased the results. However, the percentage volume composition of the total phytoplankton in the stomach contents was estimated as before. This procedure was also adopted for the detrital composition.

Zooplankton, *Microcystis* and *Phacus* were identified and enumerated as for the water samples. It was decided to treat *Microcystis* and *Phacus* separately because of their size compared to the other algal species (approximately x900 and x15 respectively, comparing biovolumes with the largest of the other species).

The percentage volume contributions were calculated using the numbers present in the stomach and biovolume data, assuming the total volume they represented was 100%, less

the phytoplankton plus detritus percentage volumes (obtained from visual estimates). However, the *microcystis* contribution was estimated visually when its biovolume was greater than approximately twenty percent.

During the *microcystis* bloom preliminary observations revealed that the water and stomach samples were dominated by this species. *Nitzschia* was present but occurred in negligible numbers, as did the zooplankton species.

Using the stomach content and water sample data for the study of feeding habit with ontogeny it was possible to calculate feeding selectivities for the different fish size classes. The selectivity on a particular food-type was calculated using the electivity index of Ivlev (1961) :

$$\text{Electivity, } E = (r - p) / (r + p)$$

where r is the proportion of a food item in the diet and p is the proportion in the environment.

Table 9. Phytoplankton biovolume data

Genus	Shape	Volume (μm^3)	Volume from literature (μm^3) (Ref)
UNICELLS			
Cryptomonas	ell	1849±182	2500 (1), 1950-3750(2) 1658(3)
Trachelomonas	sph	1127±43	
Nitzschia	cyl	575±51	360, 402 (3)
Cyclotella	cyl	2345±164	400-10000(1), 540-980(2) 1600(3), 200-1000(5)
Chlorella & Chlorococcum	sph	614±180	200(1), 33(2), 30(3), 30(5)
Chroococcus	sph	563±67	60-150(5)
Ankistrodesmus	bicon	33.3±8.9	30(2), 26(3)
Phacus	ell	33184±4608	
COLONIES			
Scenedesmus	cubic	817±133	860(4), 1000(1), 710(3), 30-200(5)
Oocystis	ell	827±141	400(1), 640-2960(3), 200-300(5)
Crucigenia	cubic	1605±276	605(3)
Phytoconis	sph	1504±205	
Merismopedia	plate	734±120	
Westella	sph	491±49	
Microcystis	sph cyl	$2.4560 \pm 0.3908 . 10^6$ $1.7345 \pm 0.1969 . 10^6$	$4.2 (0.034 - 97) . 10^6$ (2), 100000(1), 20000(4)

Abbreviations of geometric figures :
ell - ellipsoid; sph - sphere; cyl - cylinder; bicon - two cones fused at there bases

References: (1) Findenegg, Nauwerck in Vollenweider (1974).
(2) Reynolds (1984).
(3) Bellinger (1974).
(4) Dokulil *et al.* in Schiemer (1983).
(5) Berman & Pollinger (1974). (volumes given are for individual cells).

Table 10. Zooplankton biovolume data.

Genus	Shape	Volume (μm^3)	Volume from literature (μm^3)
Rotifera	*ell*	$8.86 - 11.49.10^5$	
Copepoda	*mod.ell* +	$7.77 - 9.90.10^6$	$1.80 - 18.03.10^6$ (1)
Nauplii	*mod.ell* +	$5.28 - 6.82.10^5$	$0.33 - 2.79.10^5$ (1)
Cladocera	*ell*	$11.27 \pm 2.65.10^6$	$0.90 - 25.10.10^6$ (1)

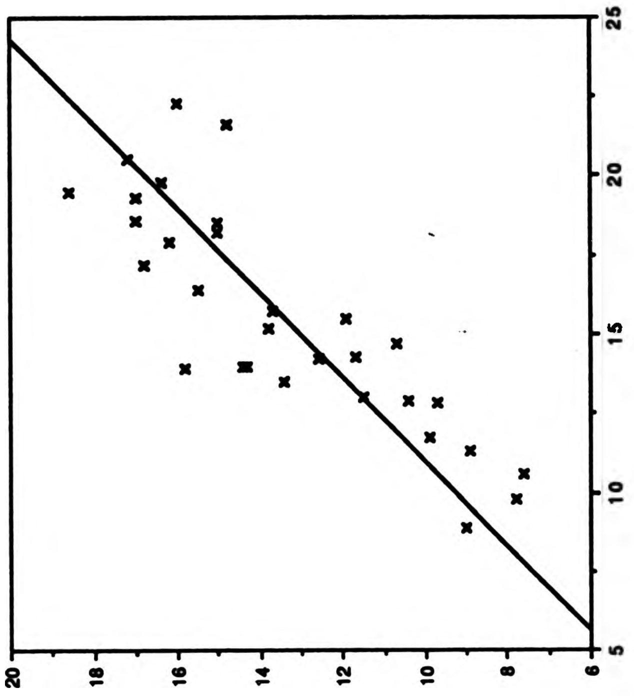
Abbreviations of Geometric Figures : ell - ellipsoid; mod.ell - modified ellipsoid
+ from Lawrence et all. (1987)

References: (1) Lawrence et al (1987)

Table 11. Comparison of estimated and calculated percentage volume contributions

	Percentage volume : estimated (calculated)			
Water Sample	Phytoplankton	Zooplankton	*Microcystis*	Detritus
A 9.11.87	75 (72.6)	20 (22.4)	+	5
A 15.11.87	45 (47.6)	10 (7.4)	+	45
C 14.11.87	65 (58.5)	5(7.0)	15 (19.6)	15
C 17.11.87	65 (65.4)	25(24.6)	+	10

Fig. 13. The relationship between empty stomach dry weight (mg) and fish wet weight (g).


Empty stomach dry weight (mg)
20
18
16
14
12
10
8
6
Fish wet weight (g)
5
10
15
20
25

4.3 Results

4.3.1 Water Quality

Daily chlorophyll 'a' and pH values in the cages and the pond are shown in figures 14a-d. The increase in chlorophyll 'a' to concentrations of 200 - 400 mg m^{-3} corresponds with the occurence of the *Microcystis* bloom, first apparent on 23.11.87 .

From readings taken during the diurnal trials it was apparent that D.O. values were consistently at their lowest at 0600 hours and increased during the day until 1800 hours after which they fell to the morning low (fig. 15a-c). This pattern was the same for the water temperature (fig. 15a-c).

The pH of the water was approximately 7 at the start of the field trial but rose to 9 - 10 by the end of the study when the pond was dominated by the *Microcystis* bloom.

4.3.2 Diurnal Feeding Studies

The first diurnal study was an investigation of *O. niloticus* of approximately 80mm S.L.. Initially, 0900 hours, the stomachs were empty. At 1200 hours all the fish stomachs examined contained caddis fly larvae only. The mean number present in each stomach was 426± 34 (n=3) and the stomach fullness index (SFI) was five. The dry weight of the stomach contents was 46.3 ± 11.4 mg (n=5). At 1500 and 1800 hours the stomachs contained the digested remains of the caddis larvae. The SFI's had decreased to 3 and 1 respectively and the dry weight of the contents to 15.2 ± 9.3 mg (n=5) at 1500 hours. By 2100 hours the stomachs were empty and remained so until 0900 hours the following day. At 1200 hours on the 7.11.87 the contents were as outlined in table 12. The results are represented graphically in figure 16.

The data collected from the second feeding trial of 80mm S.L. *O. niloticus* is given in table 13. It was only possible to quantify the diet compostion at 2100 hours (table 14) due to the different aims of the diurnal study. However, during the period when the fish were feeding the stomach contents were always a green/brown colour indicating that there was no selective feeding on a predominantly animal food type. In the first trial the colour of the stomach contents was cream when feeding on caddis larvae and green/brown when feeding on mixed food types at 1200 hours. The feeding pattern during this trial was very different to that of the first study. At 1200 hours there was no apparent synchronization of feeding with SFT's ranging from 0 to 4. At 1500 hours the fish had similar SFT's and stomach content dry weights. The fish remained feeding until 2400 hours. However, stomach fullness never reached the levels of the previous trial when feeding vorasciously on caddis larvae.

The pH of the stomach was plotted against the stomach content dry weight, figure 17. The curve was best fitted using a logarithmic regression

$$Y = -1.48 \log_e(1 + X) + 6.21 \ (r^2 = 0.92)$$

where Y is the stomach pH and X the stomach content dry weight (mg).

The third diurnal study concerned fish of 35mm S.L. Results are given in table 15. It is apparent that the feeding pattern was similar to the second feeding study of 80mm S.L. fish. The composition of the stomach contents show no particular trends with high temporal variation being shown for all food types. The colour of the stomach contents was green to green/brown except at 1800 hours when they were cream. This corresponds to a period of feeding on predominantly zooplankton (= 58% biovolume composition). The results are represented graphically in figure 18.

4.3.3 Ontogeny of feeding habits

Results are given in table 16 and figure 19. For each size class the zooplankton

contribution is very small (2.4 to 7.2%) whereas detritus and phytoplankton play a major role in the diet. The percentage of phytoplankton in the diet is increased in the three larger size classes with the *Microcystis* contribution increasing with increased size of fish. Table 17 gives the algal species found in the stomachs of the four size classes of fish. The detrital component is very large, 80% in the 35mm S.L. fish but decreases to ≈ 40% for the larger fish. The results for the Ivlev's electivity indices are shown in figure 20. The pattern is similar for both 65 and 80mm S.L. fish with the indices for the 50mm S.L. fish being a transition between the 35mm S.L. size class and the two larger size classes.

A prominent feature of all feeding trials was the complete absence of *Cryptomonas* sp. from the stomach samples despite its common presence in the water column, contributing from 9.1 - 37.6% of the phytoplankton biovolume.

The stomach contents of both 40 and 80mm S.L. fish during the *Microcystis* bloom were dominated by this algae. Zooplankton species and *Nitzschia* were present but occurred in very low numbers. Stomach contents were bright green in colour.

4.3.4 Histology and Ultrastructure of the pharyngeal feeding apparatus

The ultrastructure and development of the pharyngeal region, as revealed by SEM, was the same as that of previous studies (Northcott & Beveridge, 1988 and Beveridge *et al*, in press).

The histological examination revealed a few differences between the filter feeding apparatus of fish studied in Thailand and those in the previous study (Northcott & Beveridge, 1988). The developmental study revealed that the mucus cells of the gill arch and gill rakers became prevalent in fish ≥ 50mm S.L. It was also apparent that the staining properties of the large clavate mucus cells were different.

In fish 35 - 65mm S.L. these cells stained purple/pink following alcian blue (pH 2.5)/PAS and bright pink following alcian blue (pH1.0)/PAS indicating a predominance of neutral mucosubstances but with some acidic mucosubstances present. The clavate cells of the 80mm S.L. fish tended to have an increased proportion of acidic mucosubstances, staining purple to blue following alcian blue (pH 2.5)/PAS and bright pink to pale pink following alcian blue (pH 1.0)/PAS.

The results of the histological studies on fish sampled during the diurnal trial showed that there was no change in staining properties or morphology of the mucus cells during the feeding periods studied. However, the large clavate mucus cells of the 80mm S.L. size group showed a predominance of neutral mucosubstances as shown by the 35 - 65mm S.L. fish of the ontogenic study. The mucus cells of the 35mm S.L. fish had the same staining properties as above.

The histology of the mucus cells of the 80mm S.L. fish during the *Microcystis* bloom were the same as for this size class in the ontogenic study i.e. an increased proportion of acidic mucosubstances. However, the clavate mucus cells of 40mm S.L. fish during the bloom showed an increased proportion of acidic mucosubstances staining as for the larger size class. Purple/pink to blue following alcian blue (pH 2.5)/PAS and bright to pale pink following alcian blue (pH 1.0)/PAS.

The mucus cells in the sockets of the mandibular and pharyngeal teeth of all fish studied stained blue following alcian blue (pH 1.0)/PAS and, therefore, contained a sulphated glycoprotein.

4.3.5 Fish Growth

Despite no supplementary feeding during the experimental period there was good growth of the fish. The data from the sample weighings are given in table 18.

Table 12 Percentage volume composition of the diet of 80mm S.L *O. niloticus* at 1200 hours, 7.11.87

FOOD ITEM	% OCCURRENCE
Rotifera	4.2
Copepoda	+
Naupilii	0.6
Cladocera	1.1
Microcystis	2.1
Phacus	22.0
Phytoplankton	2 0
Detritus	5 0
Total PHYTOPLANKTON	44.1
Total ZOOPLANKTON	5.9

Table 13 Stomach Fullness Indices, SFI; stomach content dry weight, SCD (mg) and stomach pH during the second diurnal feeding study on ≈ 80mm S.L. *O. niloticus*.

Time	Parameter	*O. niloticus* specimen 1	2	3	4	5
	SFI	0	0	3	3	4
1200	SCD	-	-	12.4	17.8	19.6
	pH	5.68	5.00	1.90	2.10	1.60
	SFI	3	3	3	3	4
1500	SCD	8.2	15.0	12.7	13.3	17.1
	pH	1.50	2.23	2.32	1.70	1.70
	SFI	3	3	3	4	4
1800	SCD	23.8	15.9	16.7	40.0	26.5
	pH	1.00	1.50	1.94	1.48	1.64
	SFI	3	3	3	3	4
2100	SCD	12.7	9.5	12.2	34.4	36.9
	pH	2.04	2.25	1.72	1.41	1.04
	SFI	0	3	4	4	4
2400	SCD	-	14.8	25.2	36.5	44.9
	pH	6.70	1.52	1.54	1.57	1.37
	SFI	0	0	0	0	2
0300	SCD	-	-	-	-	*
	pH	6.74	5.51	5.60	6.20	1.76
	SFI	0	0	0	0	0
0600	SCD	-	-	-	-	-
	pH	6.92	6.77	7.03	7.03	6.95
	SFI	0	0	0	0	0
0900	SCD	-	-	-	4.0	3.9
	pH	5.30	6.34	6.25	5.44	3.62

Table 14. Percentage volume composition of the diet of 80mm S.L *O. niloticus* at 2100 hours, 9.11.87.

FOOD ITEM	% OCCURRENCE
Rotifera	3.8
Copepoda	1.8
Naupilii	0.6
Cladocera	0.8
Microcystis	11.3
Phacus	1.7
Phytoplankton	30
Detritus	50
Total PHYTOPLANKTON	43.0
Total ZOOPLANKTON	7.0

Table 15. Range of SFTs and percentage volume composition of the diet during the diurnal feed trial of small 35mm S.L. *O. niloticus*.

Food Type	SFI	3	0	1-3	3-4	5	3-5	1-3	0
	Time	0900	1200	1500	1800	2100	2400	0300	0600
		Percentage occurrence							
Rotifera		3.8	-	11.2	1.9	18.4	27.9	16.1	-
Copepoda		-	-	12.8	29.9	0.7	1.1	2.0	-
Nauplii		0.2	-	0.2	0.2	+	0.1	0.1	-
Cladocera		-	-	4.3	14.5	0.8	0.9	1.8	-
Microcystis		13.5	-	+	2.0	40	50	60	-
Phacus		2.5	-	1.5	11.6	+	+	+	-
Phytoplankton		20	-	15	10	10	15	10	-
Detritus		60	-	55	30	30	5	10	-
Total PHYTOPLANKTON		36.0	-	16.5	23.6	50.0	65.0	70.0	-
Total ZOOPLANKTON		4.0	-	28.5	58.1	19.9	30.0	20.0	

Table 16. Percentage volume composition of the diets of four size classes of *O. niloticus*

Food Type	size, mm, S.L.			
	35	50	65	80
	Percentage occurrence			
Rotifera	1.5	1.5	3.5	3.4
Copepoda	3.5	0.7	2.5	1.7
Nauplii	+	0.2	0.1	0.2
Cladocera	0.2	+	1.1	0.5
Microcystis	4.9	16.3	22.1	23.6
Phacus	+	1.3	0.7	0.6
Phytoplankton	10	40	30	25
Detritus	80	40	40	45
Total PHYTOPLANKTON	14.9	57.6	52.8	49.2
Total ZOOPLANKTON	5.1	2.4	7.2	5.8

Table 17. Algal genera found in the stomach contents of the four size classes of *O. niloticus* during the ontogenetic study.

Chlorophyta

Ankistrodesmus sp.
Actinastrum sp.
Chlorella sp.
Chlorococcum sp.
Crucigenia sp.
Dictyosphaerium sp.
Oocystis sp.
Pediastrum sp.
Phytoconis sp.
Scenedesmus sp.
Tetraedron sp.

Cyanophyta

Chroococcus sp.
Merismopedia sp.
Microcystis aeruginosa
Westiella

Bacillariophyta

Cyclotella sp.
Nitzschia sp.

Euglenophyta

Phacus sp.
Trachelomonas sp.

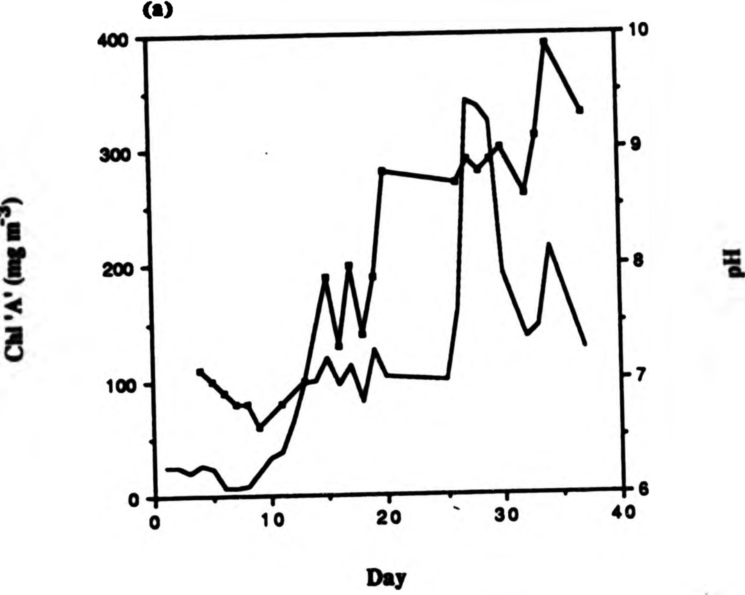
Table 18. Data from sample weighings (n=30)
* n = 22; + n = 40.

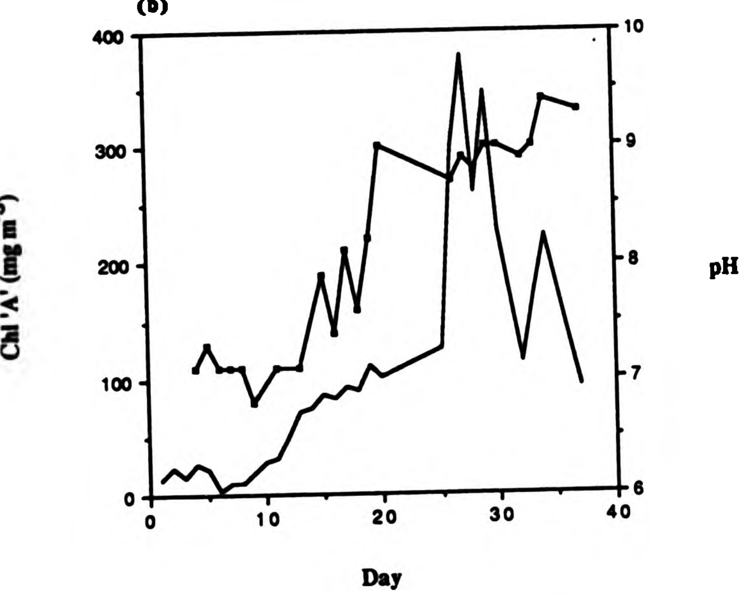
Cage	wet weight (g)				MSG
	27.10.87	5.11.87	30.11.87		
A	12.97 ± 3.05	15.12 ± 3.06	33.19 ± 5.56	*	156
B	6.95 ± 2.15	7.92 ± 1.64	21.57 ± 4.21		210
C	2.66 ± 1.05	3.38 ± 1.74	7.72 ± 4.04	+	190

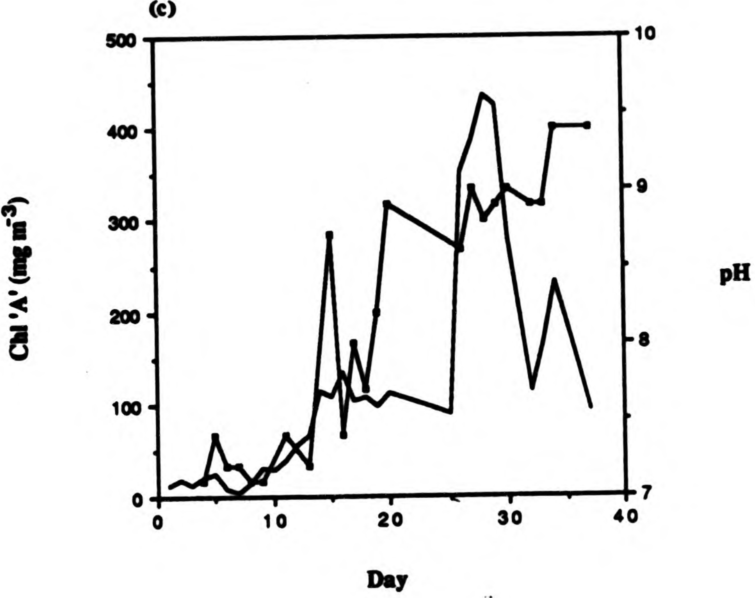
MSG Monthly specific growth rate as % initial weight.

Fig. 14. Daily chlorophyll 'a' (mg m^{-3}) and pH values in the cages and pond.
(a) Cage A
(b) Cage B
(c) Cage C
(d) Pond.

—	Chl 'A'
—•—	pH


(a)
Chl 'A' (mg m-3)
pH
Day
0
10
20
30
40
100
200
300
400
6
7
8
9
10


(b)
Chl 'A' (mg m-3)
pH
Day
0
10
20
30
40
100
200
300
400
6
7
8
9
10


(c)
Chl 'A' (mg m-3)
pH
Day
0
100
200
300
400
500
7
8
9
10
0
10
20
30
40

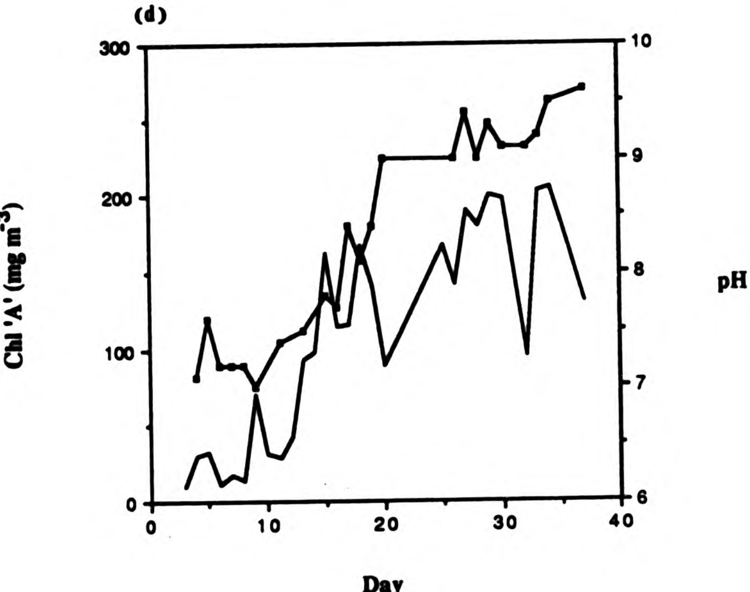
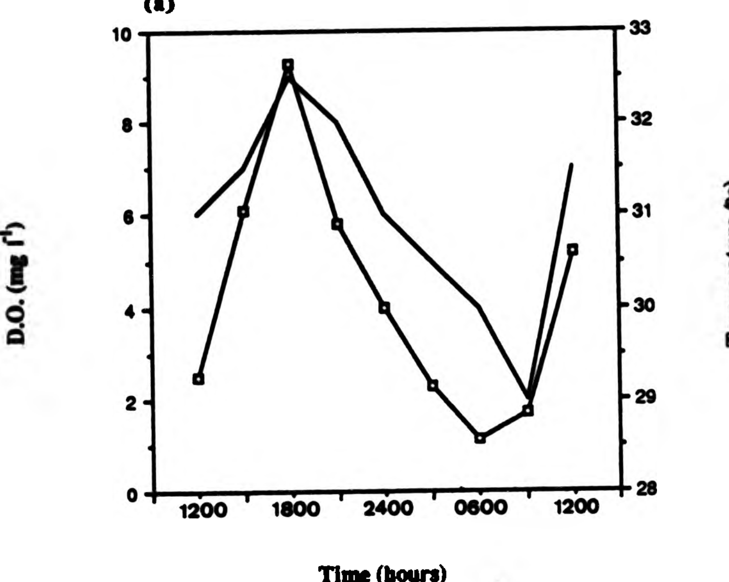
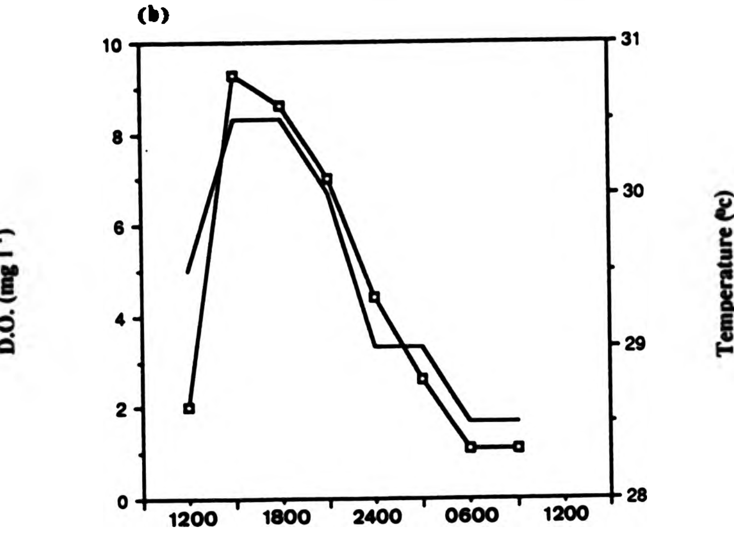

(d)
Chl 'A' (mg m-3)
pH
Day
0
100
200
300
6
7
8
9
10
0
10
20
30
40

Fig. 15. Dissolved oxygen, D.O. (mg l^{-1}) and temperature (°c) values during the diurnal feeding trials.
(a) Cage A, 6-7.11.87.
(b) Cage A, 9-10.11.87.
(c) Cage C, 17-18.11.87.

-□- D.O.
— Temperature


(a)
D.O. (mg l-1)
Temperature (ºc)
Time (hours)
1200
1800
2400
0600
1200


(b)
D.O. (mg l-1)
Temperature (ºc)
Time (hours)
1200
1800
2400
0600
1200

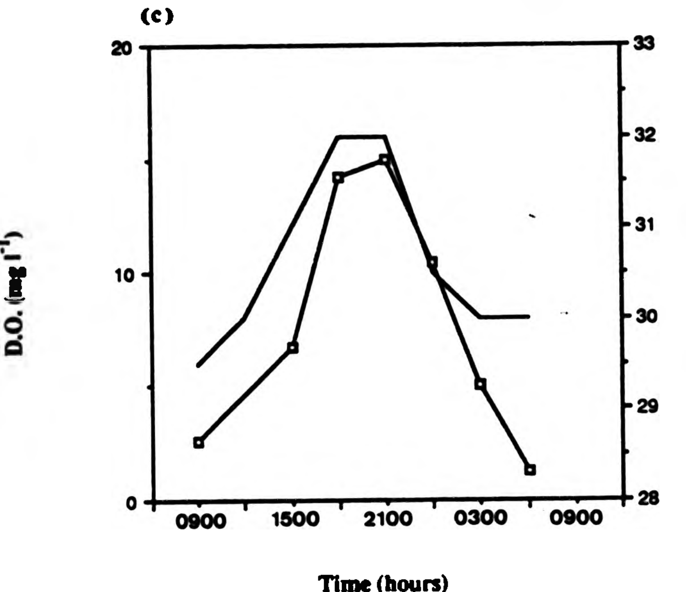

(c)
20
10
0
33
32
31
30
29
28
0900
1500
2100
0300
0900
D.O. (mg l-1)
Temperature (°c)
Time (hours)

Fig. 16. Percentage volume composition of the diet of 80 mm S.L. *O. niloticus* at 1200 hours, 7.11.87.

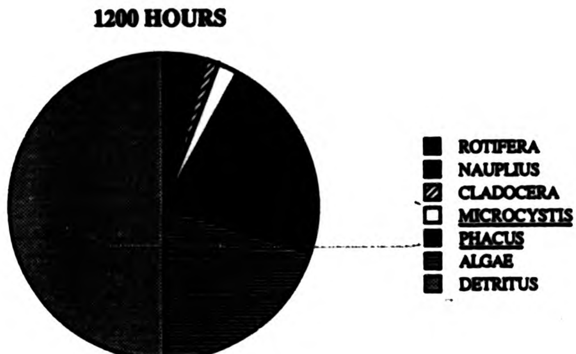
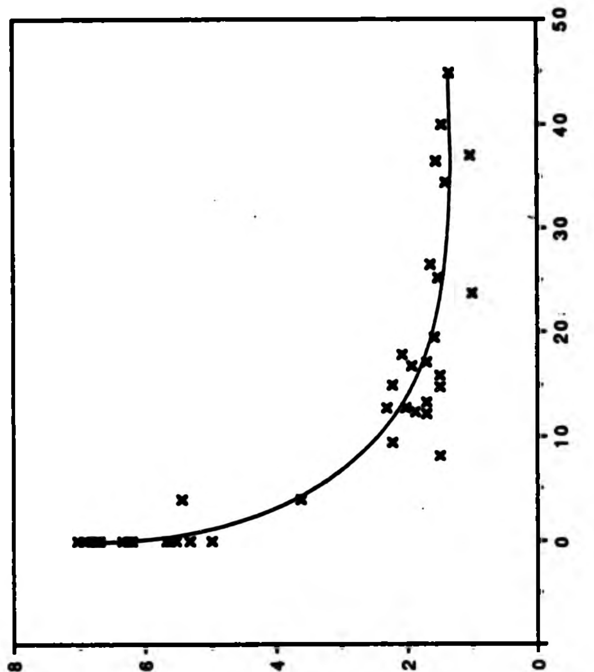

1200 HOURS
ROTIFERA
NAUPLIUS
CLADOCERA
MICROCYSTIS
PHACUS
ALGAE
DETRITUS

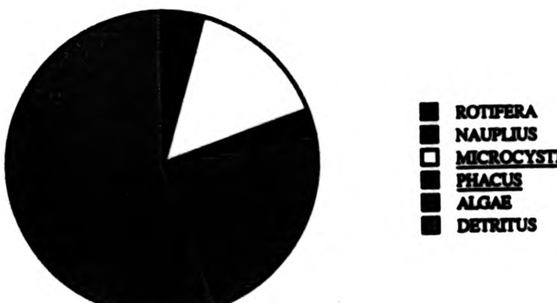
Fig. 17. The relationship between stomach pH and stomach contents dry weight (mg).



Stomach contents pH
Stomach contents dry weight (mg)
0
2
4
6
8
0
10
20
30
40
50

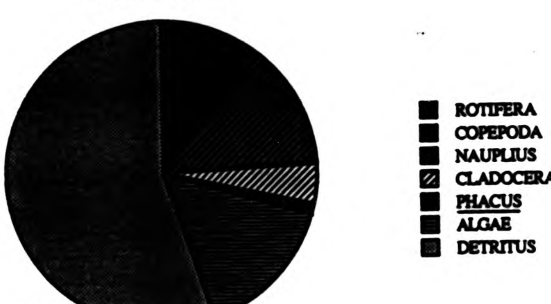
Fig. 18. Percentage volume composition of 35 mm S.L. *O. niloticus* during the diurnal feeding trial.

0900 HOURS

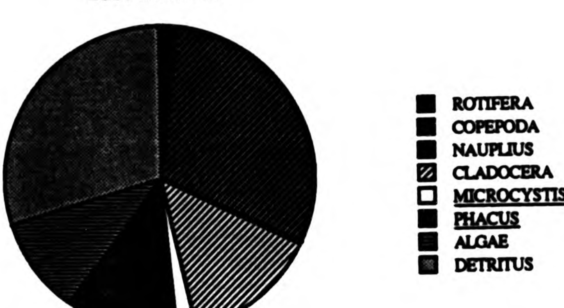


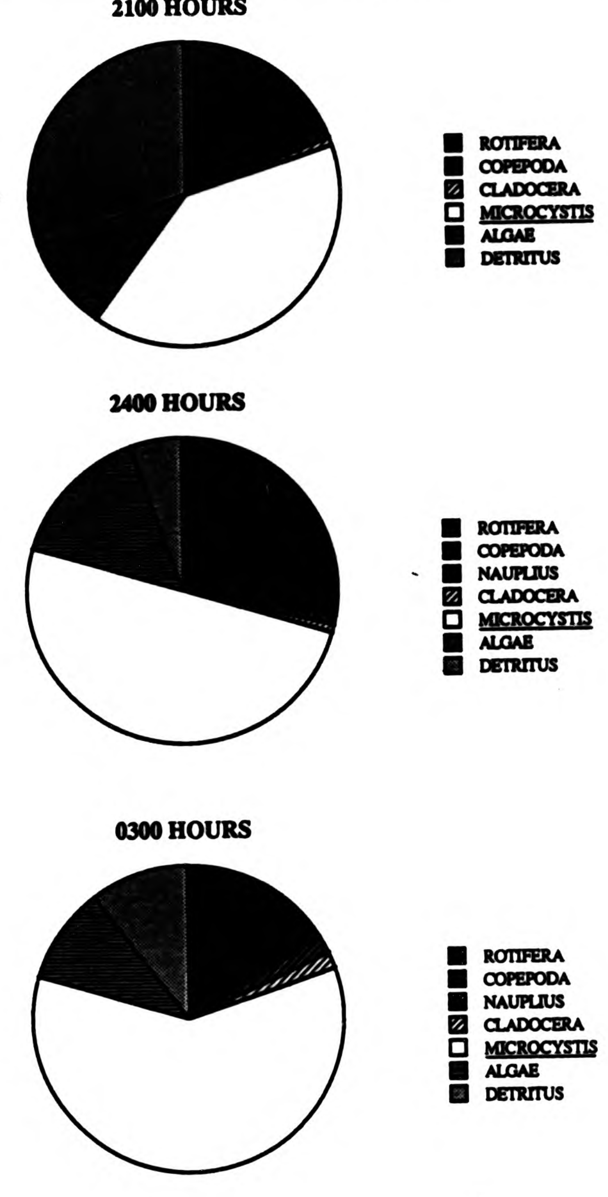
1200 HOURS - Stomachs empty

1500 HOURS



1800 HOURS

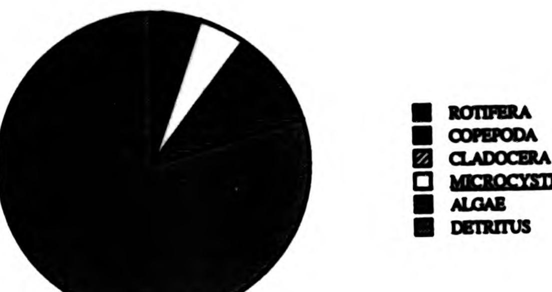




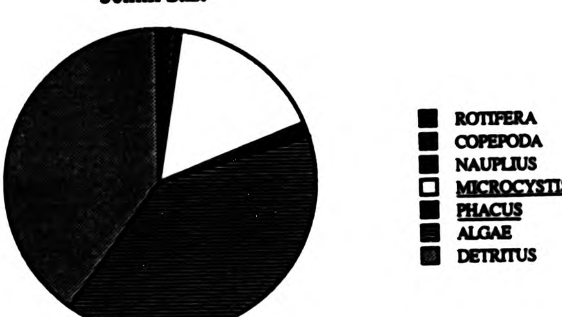
0600 HOURS - Stomachs empty

Fig. 19. Percentage volume composition of the diets of four size classes of *O. niloticus*.

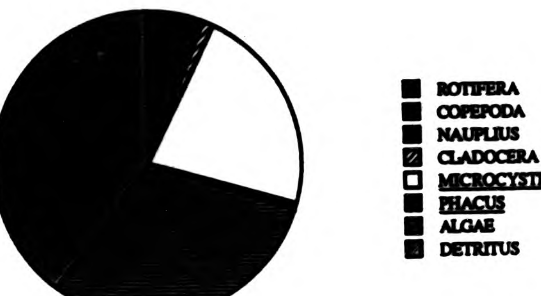
35mm S.L.



50mm S.L.



65mm S.L.



80mm S.L.

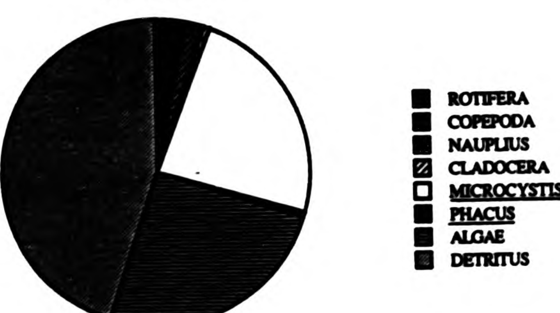
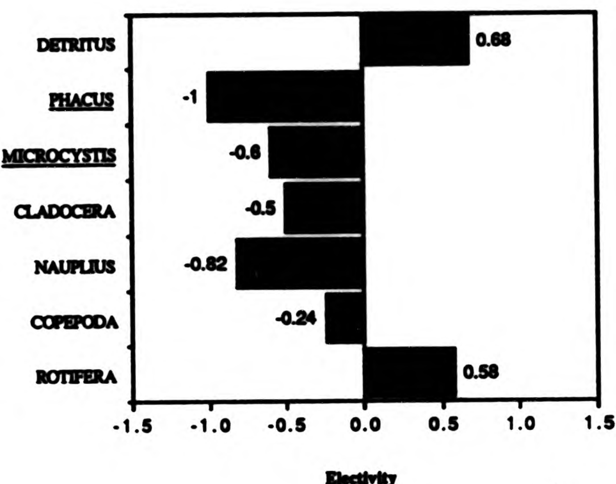
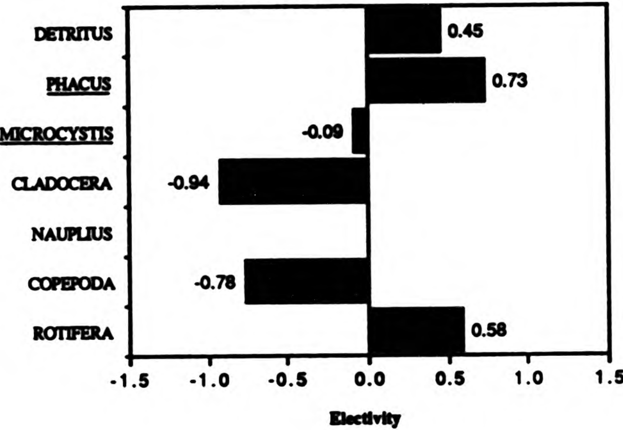
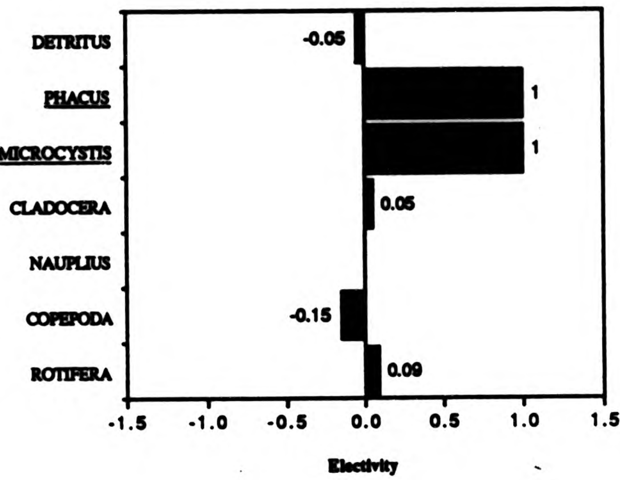


Fig. 20. Ivlev's electivity indices for the four size classes of *O. niloticus*.

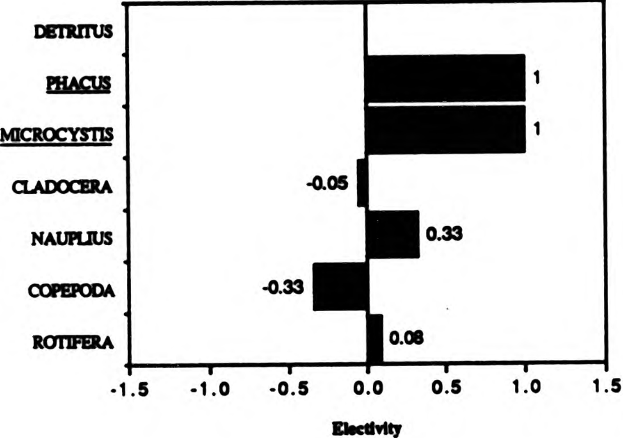

35mm S.L.
DETRITUS
PHACUS
MICROCYSTIS
CLADOCERA
NAUPLIUS
COPEPODA
ROTIFERA
0.68
-1
-0.6
-0.5
-0.82
-0.24
0.58
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Electivity

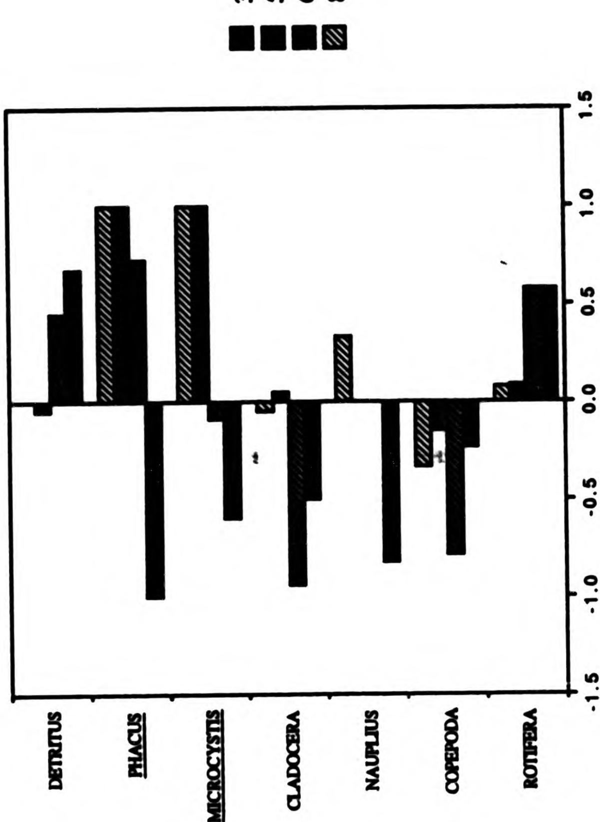

50mm S.L.
DETRITUS
PHACUS
MICROCYSTIS
CLADOCERA
NAUPLIUS
COPEPODA
ROTIFERA
0.45
0.73
-0.09
-0.94
-0.78
0.58
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Electivity

65mm S.L.



80mm S.L.




DETRITUS
PHACUS
MICROCYSTIS
CLADOCERA
NAUPLIUS
COPEPODA
ROTIFERA
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Electivity
35mm SL
50mm SL
65mm SL
80mm SL

4.4 Discussion

The data presented in this chapter reveals the diverse feeding habits of *O. niloticus*. De Silva *et al*. (1984) proposed that the diet of populations devouring more than 50% of detritus, plant and animal material should be considered to be detritivorous, herbivorous and carnivorous respectively. From the results presented here it can be seen that both small and large *O. niloticus* had dietary habits corresponding to all of the above modes at some time during the study. De Silva *et al*. (1984) confirmed the versatile dietary habits of *O. mossambicus* in connected water system in Sri Lanka and Maitipe & De Silva (1985) investigating *O. mossambicus* in twelve man-made lakes, Sri Lanka, showed that none of the populations maintained either a detritivorous, phytoplanktivorous or a zooplanktivorous habit through the year.

During this study, although at times the dietary spectrum was very wide, the fish were also observed to feed predominantly on single prey items. Yashouv & Chervinski (1960) studying the feeding of *O. niloticus* in ponds in Israel, found that the fish would feed on the wide range of food items in the water even when the prey were offered on their own, as a single diet. In this study, the ingestion of single prey items was selective, as observed when the large *O. niloticus* fed on caddis fly larvae. However, ingestion of a single prey item can be due to the uniform composition of food in the environment. When small and large *O. niloticus* ingested predominantly *Microcystis* during the bloom of this algae the *Microcystis* colonies formed nearly 100% of the food biomass in the cages. Gophen (1980) reports that the intestines of *S. galilaeus* caught in the summer in Lake Kinnert were full of *Peridinum*. Spataru (1976) states that *Peridinium* blooms during March to April sometimes forming more than 95% of the phytoplankton biomass. However, *Peridinium* was also found in the guts throughout the year, even when the algae was sparse, indicating selective feeding by *S. galilaeus*.

This study demonstrated that in the pond cage situation under investigation the ontogenetic

change in diet, documented for *O. niloticus* in its natural habitat (Trewavas, 1983), is not a definite event. Large *O. niloticus* were at times both carnivorous and detritivorous. However, fish smaller than the 'switch over' length held in water dominated by suspended *Microcystis* were observed to feed on this alga. The changes in diet within natural populations, however, appear to be related to changes in habitat. Moriarty *et al.* (1973) observed changes in *O. niloticus* to a phytoplanktivorous diet corresponding with a migration into deeper water. Yashouv & Chervinski (1961) showed that this switch in *O. niloticus* also corresonded to changing habitat, even within ponds.

During the study on the development of the feeding ecology it was possible to examine the feeding selectivities of the fish of different size classes. All size classes showed positive selection for rotifiers and negative selection for copepods (Fig. 20). This agrees with the work by Drenner *et al.* (1984b) which showed that large *O. aureus* (125-190 mm S.L.) suppressed (inhibited the growth of) populations of the rotifer *Keratella* sp. but enhanced (stimulated the growth of) copepodid and adult stages of the copepod *Diaptomus*. The selection for copepod nauplii (Fig. 20) changed from negative electivity in 35 mm S.L. *O. niloticus*, through no specific electivity for mid-length fish, to positive selection at 80 mm S.L. This agrees with Drenner *et al.* (1982) investigating the feeding of *S. galilaeus* in zooplankton assemblages. They found that fish of 20-42 mm S.L. feeding as particulate feeders had negative selection for copepod nauplii and filter feeding fish had positive electivity. In the study of Drenner *et al.* (1982) fish greater than 62 mm S.L. were obligate filter feeders. However, they found positive selection for cladocera for both particulate and filter feeding fish. In this work there was a tendancy of negative electivity for cladocera (Fig. 20). However, the work of Drenner *et al.* (1982) was for fish feeding solely on zooplankton assemblages and the suction dynamics during the filter feeding process may have been stronger.

The two larger size classes studied in this work had strong positive selection for both *Microcystis* and *Phacus*. Both these algae had large biovolumes relative to the other

phytoplankton. Moriarty *et al.* (1973) showed that *O. niloticus* had marked positive selection for the large colonial and filamentous algae, *Microcystis*, *Lynbya* and *Melosira*. This was confirmed for *O. mossambicus* by Dokulil (1983). Vinyard *et al.* (1988) using *S. galilaeus* and *O. aureus* in mesocosm (small, enclosed ecosystems) experiments observed that both species suppressed *Peridinium*. Like *Phacus*, *Peridinium* is a flagellate and has a comparable cell volume, 31000μm^3.

It must be stressed that the selectivities from the ontogenetic study should be interpreted with the utmost caution as rapid lysis of certain organisms (Doud, 1974, cited in Hyslop, 1980; Gannon, 1976; Berg, 1979) may bias the results. Moreover, the variation in the diets observed during the diurnal trial of this study suggests the ability to change selectivity. The smaller fish examined in the ontogenetic study showed a marked positive electivity for detritus (Fig. 20). However, the low occurrence of detritus in the diet at 2400 hours (Table 15) suggest negative selection of detritus with a switch to positive selection of *Microcystis*.

The most striking anomoly in the results was the complete absence of *Cryptomonas* sp. from the stomach contents. This could have been due to very rapid lysis of the cells in the low pH of the stomach. However, during microscopic examination of the stomach contents, all algae, except *Cryptomonas* sp., were clearly observed. This tends to disprove the lysis theory, as *Cryptomonas* sp. have no obvious features which make them susceptible to rapid lysis. There could be a negative selection by *O. niloticus* or some mechanism by which these algae can escape the 'capture' process. Drenner *et al.* (1984a), in pond grazing trials, showed that the gizzard shad, *Dorosoma cepedianum*, enhanced *Cryptomonas* together with certain other algal species.

Throughout the experimental period, despite the presence of a varied phytoplankton, zooplankton and detritus assemblage in the pond, it was noticeable that detritus was a major component of the diet in all the fish sizes studied. Bowen (1979) working on

O. mossambicus in Lake Sibaya, S. Africa, showed that both juvenile and adult fish maintained a uniform detrivorous diet. The adults suffered from severe malnutrition due to the decreased nutrional quality of the detritus with increasing depth. Juveniles fed on benthic detrital aggregate from nearshore sand terraces, whereas adults fed on benthic detritus in deeper, offshore waters. Detritus in water shallower than approximately 0.8 m was sufficient for growth whereas detritus in water deeper than approximately 1.2 m led to malnutrition due to the ratios of digestible protein to digestible energy showing a highly significant inverse relationship to increasing depth. These ratios indicated that juvenile diets contained adequate protein to support growth, but adult diets would have a protein deficiency. In the present study the cages were held in the top 0.9 m of the pond water column, where detrital quality was probably highest and, therefore, beneficial to both the small and large size classes of *O. niloticus*.

Bowen (1979) substantiated the observations of Lowe - McConnell (1975) stating that detritivorous fish are dietary specialists with uniform rather than varied diets. This may be due to environmental conditions, as De Silva *et al*. (1984) found that although the diet of *O. mossambicus* could be detritivorous it also ranged from herbivory to total carnivory. In the present study although *O. niloticus* was shown to exhibit detritivory its diet was also shown to be extremely variable.

The importance of detritus in the diets of phytoplanktivorous fish might be explained by the suggestions of Friedland *et al*. (1984) who considered that its presence would enable grazing fish to retain smaller particles than could be retained in clear water, due to the clogging action of detritus on the filtering apparatus. Detrital enhancement of filtration has been suggested for cladocerans (Porter *et al*., 1983).

The varied diet of *O. niloticus* recorded in these field studies was accompanied by a wide range of diurnal feeding patterns. the feeding periods of fish in this study may have been related to the diet quality and composition. When the large fish fed voraciously on the

caddis fly larvae the feeding period was very short. However, when ingesting a mixed diet the feeding period extended from midday until midnight (tables 13 & 15). This latter pattern was also observed for the small fish. However, a reduced feeding period was not observed despite a higher proportion of zooplankton in the diet at 1800 hours (Table 15).

Moriarty & Moriarty (1973) found that *O. niloticus* began to feed shortly before dawn and fed continuously until about dusk. The diurnal feeding of the phytoplanktivorous *S. g. multifasciatus* was shown to be related to a diel migration (Whyte, 1975). The fish migrated to depths of up to 30 m during the day and rose to the surface tofeed between 1800 and 0500 hours. The feeding of *O. niloticus* on phytoplankton (Getachew, 1987) was studied by overnight gill-netting which again suggests night-time feeding. Hofer & Schiemer (1983) found that the phytoplankton and mineral sediment diet of juvenile *O. mossambicus* was taken during a continuous diurnal feeding cycle.

During the field experiments the ultrastructure and histology of the pharyngeal apparatus were examined in relation to development and diet. The ultrastructural development, observed by SEM, was the same as observed in laboratory-held populations. However, there were some gross histological differences.

The uncommon occurence of mucus cells in the anterior pharyngeal chamber and their increased occurence in the posterior pharynx of small (≈ 35 mm S.L.) *O.niloticus* suggests a particulate feeding mode. The prevalence of mucus cells throughout the pharyngeal chamber of fish ≥ 50 mm S.L. probably corresponds to the change to filter feeding. This size class is the point at which the feeding mode has been observed to change by various workers (Moriarty *et al.*, 1973; Trewavas, 1983).

The histological investigation of the pharyngeal filtering apparatus of fish examined from the diurnal trials show that the size, shape and staining of the mucus cells with alcian blue / PAS was consistent throughout the 24 hour period. This suggests that mucus is always

produced irrespective of whether ingestion is occurring or not and indicates that another mechanism which prevents ingestion must be occurring. Such a mechanism could be the muscular contraction of the oesophagus or the adduction of the pharyngeal pads.

There were no differences in the histochemistry of the mucus cells during the diurnal trials. However, there was an increase in the acidic mucosubstances when both small and large *O. niloticus* consumed a phytoplanktivorous diet of *Microcystis*.

Similar mucus histochemistry of the feeding structures in other aquatic animals with comparable feeding habits have been reported. The Atlantic menhaden, *Brevoortia tyrannus*, is a pelagic filter feeder primarily consuming phytoplankton (June & Carlson, 1971). However, zooplankton (Richards, 1963) and detritus (Darnell, 1958) have also at times been found as major constituents in the stomachs. Friedland (1985) demonstrated that the mucus cells of the gill arches of *B. tyrannus* produce both neutral and acidic mucosubstances. He proposed that the acidic glycoprotein was more viscous and used to move larger food particles, whereas the less viscous neutral mucus was involved in the transport of smaller food particles.

In this study the presence of predominantly neutral mucosubstances in the pharyngeal apparatus of *O. niloticus* feeding on a varied diet may be important in the retention and transport of the smaller food items. However, when feeding on the large colonial *Microcystis*, the handling characteristics may require a more viscous, acidic mucosubstance.

Evidence suggests that the food of larval lampreys consists mainly of microscopic organisms, especially diatoms and fine particulate detritus (Schroll, 1959; Manion, 1967; Moore & Beamish, 1973). Mallat (1981) found two types of goblet cells in the lateral pharynx of the larval lamprey, *Petromyzon marinus*, secreting either acidic or neutral mucus. It was hypothesized that the alcian-nonreactive (neutral) mucus, with fewer

exposed anions, was less adherent than alcian-reactive (acidic) mucus. The alcian-nonreactive mucus cells were located lateral to alcian-reactive cells, in a region where only small, easily trapped particles are found. This suggestion of small particles being processed by neutral mucosubstances is consistent with Friedland (1985).

Similar findings have been reported for anuran larvae. Kenny (1969) showed that in twenty microphagous species the mucus cells of the pharyngeal region were PAS positive but alcian blue nonreactive. He also showed that mucus cords produced by such cells could easily entangle small particles, yeast cells (2-5 µm) and carmine particles (0.5-5 µm).

Although this study concentrated on aspects of ingestion and feeding it was possible to investigate briefly stomach pH in large (≈ 80 mm S.L.) *O. niloticus*. The stomach pH was observed to drop rapidly to values below 2.0 as the stomach filled (Fig. 17). The results from this survey agree with those of Moriarty (1973) who found that *O. niloticus* uses gastric acid below pH 2.0 to lyse blue-green algae. He found the pH values of empty stomachs ranged from 5.0 to 7.0 but during the initial feeding period the pH was in the range 2.5 to 3.0. The fish in the present study were feeding on a mixed plankton and detritus assemblage. However, Hofer & Schiemer (1983) found the pH of the mixed stomach contents of *O. mossambicus* to be very constant (1.99 ± 0.37) which agreed with the continuous feeding observed. Bowen (1976) found low stomach pH values, below 2.5, 1.5 and 1.25 being very common, which allowed *O. mossambicus* to digest the bacteria associated with the detritus in its diet.

Finally, table 18 demonstrates that despite no supplementary feeding respectable growth rates were obtained.

The monthly specific growth rate (MSG) values of this study are comparable with other MSG's calculated from growth trials of tilapias in extensive, semi-intensive and intensive

cage culture systems (Coche, 1982). In extensive cage culture there is no supplemental feeding. Semi-intensive operations involve some supplementary feed but this is relatively poor in protein (less than 10% dry weight). In intensive systems the cost of feeding gains importance and the utilization of a high initial biomass becomes necessary if production costs are to be minimized and net profits maximized. In his review of the cage culture of tilapia, Coche (1982) stated that as the culture system is progressively intensified, growth and production increase, but, in the presence of moderate to dense algal blooms, relatively high values were attained for *O. aureus* even in extensive systems. Indeed, these results were even better than for other tilapias in more intensive systems. Therefore, the extra cost of feeding should always be weighed against the production obtainable from natural foods for a given species and location. It was also stated that comparative data was lacking for *O. niloticus* which might also provide good results with algal blooms. The MSG values of this study are comparable with the higher MSG figures quoted for the intensive cage culture of *O. niloticus* (Coche, 1982). Therefore, the economies of supplemental feeding would have to be carefully considered to see if such feeding, leading to increased production, would be worthwhile.

The investigations of this chapter have concentrated on the feeding ecology of *O. niloticus* in cages held in fertilized ponds in Thialand.

The pharyngeal filter feeding apparatus, observed by SEM, was the same as laboratory-held populations; however, the histological staining of the pharyngeal mucus cells showed a predominance of neutral mucosubstances. There was a shift to a higher presence of acidic mucus during the *Microcystis* bloom. The development of the filter feeding apparatus was complete by approximately 50 mm S.L., the point at which the feeding mode has been observed to change by various workers (Moriarty *et al.*, 1973; Trewavas, 1983). The results suggest continuous pharyngeal mucus production and some other mechanism must prevent ingestion e.g. the adduction of the pharyngeal pads.

The main observation of the dietary studies was the varied feeding of both small (≈ 35 mm S.L.) and large (≈80 mm S.L.) *O. niloticus*. Both size classes were detritivorous, herbivorous or carnivorous at some time during the study. The reported switch from an omnivorous diet, when young fry, to a phytoplanktivorous diet at approximately 50 mm S.L. was not observed to be a definite event. This change in feeding in the wild is probably due to a shift in habitat. In this study all fish were held in the same environment.

The growth data of this study, calculated as MSG, are comparable with figures from the intensive culture of *O. niloticus* (Coche, 1982). This shows that *O. niloticus* could utilize the mixed plankton and detritus assemblage available in the water column. Therefore, the culture system used in this study, cages held in fertilized ponds, provides a good method for the extensive culture of *O. niloticus* from a small (≈ 30 mm S.L.) size.

Chapter 5

Discussion

"Many detours I will still follow, many fulfillments will still disillusion me.

One day, everything will reveal its meaning ... Nirvana."

Hesse, *Wandering*.

The three principal studies covered in this thesis comprise a comprehensive investigation of the filter feeding and feeding ecology of *O. niloticus*. To date, it has been a widely held view that in the natural habitat the feeding mode and diet of *O. niloticus* changes from omnivorous particulate feeding, when young fry, to phytoplanktivorous filter feeding by 60 mm T.L., ≈ 50 mm S.L. (Trewavas, 1983). The morphological study described here demonstrates that development of the filter feeding structures is complete by 40 mm S.L. (see also Plates 2, 3, 6, 11 & 12). This is further substantiated by the demonstration that small size class (≈ 40 mm S.L.) *O. niloticus* can ingest two species of blue-green algae, *A. cylindrica* and *M. aeruginosa* (Figs. 7 & 8). The mechanism involved is almost certainly by 'pump' filter feeding, as the algae ingested were too small to be entrapped by physical sieving alone. However, the variable nature of the diet of both small (≈ 35 mm S.L.) and large (≈ 80 mm S.L.) size class fish when held in cages in fertilized ponds was clearly demonstrated (Tables 12, 15 & 16 and Figs. 16, 18 & 19). The 'switch' in diet was observed not to be an obligate, discrete event (Table 16 and Fig. 19) as had previously been suggested.

The feeding mechanism described previously was stated to be one involving mucus entrapment, a theory proposed by Greenwood (1953). This was substantiated by the results of both chapters three and four. Histological examination of several specimens of *O. niloticus* from Songkhla, S. Thialand (Appendix 1) revealed similar staining

characteristics to the pharyngeal mucus cells of the Bangkok fish, despite very different water chemistry. Specimens of *O. alcalicus grahami* obtained from the soda lakes of East Africa, although having no microbranchiospines (Trewavas, 1983, substantiated by histological examination), showed similar staining and distribution of pharyngeal mucus cells (Appendix 2) as for the laboratory-held populations of *O. niloticus*, again despite widely differing water quality.

Based on the above evidence a theory of the use of mucus in the filter feeding mechanism of *O. niloticus* can be proposed. Under none of the conditions or dietary habits studied did the large or small mucus cells of the gill arches and gill rakers contain sulphated mucosubstances. However, such acidic mucus cells were demonstrated in the pharyngeal and jaw tooth sockets for all fish examined histologically. This suggests that the sulphated mucosubstances, being more viscous than sialyated or neutral mucins (Jones *et al.*, 1973; Iravani & Melville, 1974) are used as a 'heavy' lubricant in areas where abrasion of tissues and organs would be high. The raking action of the pharyngeal pads has been demonstrated clearly by Aerts *et al.* (1986) and it would thus seem necessary to protect the teeth and tissues during such abrasive action. This theory is substantiated by the SEM study of the pharyngeal apparatus of *O. niloticus* that had fed on suspended *Anabaena* (Plate 19) which shows the pharyngeal teeth to be covered by a layer of mucus.

Considering the histochemical investigation of the pharyngeal mucus cells, observed during the ultrastructural and histological investigation of laboratory-held fish, it was proposed that the mucus produced by the clavate mucus cells of the gill rakers formed a 'net' in the pores of the branchial sieve. The SEM study of the mucus entrapped *Anabaena* caught on the gill rakers and pharyngeal pads supports this, showing that the mucus of the gill arch region has a fibrous, network strucure (Plate 17), unlike the lubricant mucus of the pharyngeal pads. This net-like structure was observed in laboratory-held fish that had predominantly sialyated mucus in the clavate mucus cells.

Although the fish studied in Thailand had slightly differing histochemistry ranging from predominantly neutral to predominantly sialyated mucosubstances in the clavate mucus cells, both neutral and acidic mucins were always present in the mucus cells of the gill rakers. Therefore, it could be hypothesized that the neutral and sialyated mucins are important in the actual filtering mechanism of *O. niloticus*.

Flood & Fiala-Médioni (1981) demonstrated that the mucus 'nets' of various ascidians were both alcian blue (pH 2.5) and P.A.S. positive. In this study the gill raker mucus of all fish also contained both neutral and acidic mucosubstances. Fibrous mucus webs were also observed on the feeding structures of the larval lamprey (Mallatt, 1979) and the anuran tadpole, *Rana catesbeiana* (Wassersug, 1972). Although the mucus of *R. catesbeiana* contains no acidic glycoproteins (Kenny, 1969), its ability to form a net-like structure is probably due to the fact that it is a glycolipid (Law, 1960). The differential functioning of mucus in the feeding of various species of aquatic animals has been reported by several workers (Friedland, 1985; Sibbing & Uribe, 1985; Mallatt, 1981). However, in contrast to the current study, Sibbing & Uribe (1985) proposed that the low-viscosity sialomucines in the anterior part of the oro-pharynx aid in lubrication and the high-viscosity sulfomucins of the posterior pharynx aid in trapping small particles and aggregating them into boluses.

La Barbera (1984) and Rubenstein & Koehl (1977) have discussed the theory of particle capture in filter feeding animals. Although mucus is clearly involved in the filter feeding mechanisms of *O. niloticus* the exact method of particle capture is not known. The particles may be caught in mucus sheets in the pharyngeal chamber, comparable with the method proposed for the spined loach, *Cobitus taenia* (Robotham, 1982), and these inturn are caught by the gill rakers. Alternatively, the formation of mucus 'nets' in the pores of the branchial sieve and the presence of mucus on the gill rakers would indicate that these structures themselves act as 'sticky' filter elements. In either case the aerosol capture mechanisms of La Barbera (1984) would be applicable. (La Barbera (1984) states

that the term aerosol filtration is a misnomer in this context, for in almost all biological situations the particles are suspended in water, not air.)

The work of Drenner *et al.* (1987) appears to contradict the suggestion of the importance of the gill rakers and associated mucus in the filtering mechanism of *O. niloticus*. They found that surgical removal of the gill rakers and microbranchiospines of *S. galilaeus* did not affect either particle ingestion rates or selectivity. However, the particles used were plastic microspheres and heat-killed zooplankton, very different to using 'live' food, and their nature may have meant that the mucus produced by the mucus cells of the dorsal surface of the mouth and pharynx and the surface of the gill arches may have been sufficient to trap these particles.

As mucus is very probably involved in particle entrapment it is interesting to examine the effect of particle charge on capture. La Barbera (1978) reported that the negatively charged mucus of *O. aculeata* preferentially retained charged rather than neutral particles of the same size and density. Gesristen & Porter (1982) observed decreased capture of negatively charged particles by the cladoceran, *Daphnia magna*, as compared to neutral particles. Neihof & Loeb (1972) and Loder & Liss (1985) demonstrated that particles in suspension in fresh, marine and estuarine waters have a small negative charge. Ives (1959) showed that phytoplankton carried a net negative charge and that the algae remained electro-negative at all pH values investigated (≈ pH 2.5 to 11.5). The acidic mucus of *O. niloticus*, being negatively charged due to exposed anions (Mallatt, 1981), could preferentially trap negatively charged particles in freshwater systems due to divalent cation mediated adhesion. Costerton & Ingram (1974) postulated such a mechanism to account for the adhesion of the negatively charged bacterial glycocalyx to negatively charged surfaces.

A filter feeding mechanism based on mucus entrapment would appear to be very costly in

energy terms, especially as the histological studies of the diurnal trials at A.I.T., Bangkok, revealing no change in mucus cell morphology, indicate a continual production of mucus. A similar mechanism, based on continual mucus production has been proposed for the spined loach (Robotham, 1982). However, Darnell (1964) suggested that such mucus would largely be resorbed in the gut of the fish.

Although the feeding mechanism of *O. niloticus* may be physiologically costly, it has certain benefits in the range of food items that can be ingested, as shown by the feeding ecology studies of this thesis, carried out in Thialand. The quantitative work on the ingestion of blue-green algae by *O. niloticus* demonstrated that both small and large fish could filter feed on suspensions of *Anabaena* and *Microcystis*. However, fish held in cages in fertilized ponds in Thailand were observed to have diverse feeding habits. The wide spectrum of food items were ingested without a significant change in the histochemistry or morphology of the pharyngeal mucus cells. This may be possible due to the production of both neutral and sialyated mucosubstances by the mucus cells of the gill rakers.

A few workers have proposed that the neutral and acidic mucosubstances entrap particles of different sizes. Friedland (1985) and Mallatt (1981) both propose that neutral mucosubstances trap small particles whereas acidic mucus traps and transports larger particles. The change towards predominantly acidic mucosubstances in the pharyngeal mucus cells of *O. niloticus* during the *Microcystis* bloom may support this theory as the biovolume of the *Microcystis* colonies is relatively large compared with other algal species and the zooplankton (Tables 9 & 10). The predominant occurence of sialyated mucus in the pharyngeal region of fish sampled at Songkhla, Thailand, may also be due to the large size of the food items in the water and stomach (Appendix 1). Table 11 shows the high occurrence of phytoplankton in the water samples at the time when the gill raker mucus cells contained predominantly neutral mucus. Therefore, it may be postulated that the size

spectrum of the food items in the water column determines the relative contribution of the different mucins in the feeding mechanism. However, due to the relatively low numbers of fish studied and the great variation in water chemistry this is purely specultative. What is certain is the feeding mechanism of *O. niloticus* allows it to ingest a wide range of food items and the 'switch' in diet can be effected quickly (Table 15).

The quantitative results of chapter three were comparable with other work on anuran tadpoles (Seale & Beckvar, 1980) and *O. aureus* (McDonald, 1985). Appendix 3 shows an estimated ingestion rate of $2.72 \pm 0.18 \times 10^9 \mu m^3 g^{-1} hr^{-1}$ for *O. niloticus* feeding on a varied diet. This is comparable to an ingestion rate, at the same biovolume concentration, of $3.22 \times 10^9 \mu m^3 g^{-1} hr^{-1}$ calculated from the regression equation of small *O. niloticus* feeding on *Anabaena* (Table 8). The higher ingestion rate for the laboratory trials may be due to the fact that the fish were starved for 24 hours prior to the filter feeding trials, a trend observed by Frost (1972) and Wassersug & Hoff (1979). However, a problem with this comparison is that the fish used in the fieldwork in Thailand were consuming much larger particles - only 15% of the biovolume was phytoplankton - and may therefore have been particlulate feeding.

Often, the results of feeding curves are incorporated into simulation models of trophic dynamics (Lehman, 1976). Moriarty *et al.* (1973) carried out a laboratory study of the ingestion and digestion of algae by two species of fish and the copepod *Thermocyclops hyalinus*. These data were then used to estimate the dynamics of the algal population in Lake George, Uganda. Such an exercise seemed feasible as the diurnal feeding cycles of the species were consistent, as were the diets, all species feeding predominantly on phytoplankton. However, the fieldwork in Thailand showed that ecosystem modelling using data obtained from the laboratory feeding trials of *O. niloticus* ingesting blue-green algae may not to be worthwhile. Several examples of problem areas were demonstrated. Noticeably the diets of *O. niloticus* were varied, and 'switching' to other food items

occurred over a short period of time (Table 15). Moreover, the diurnal feeding rhythms were inconsistent. Basic ecological modelling using laboratory generated data may only be feasible given that algal species used in the trials were the same as or comparable to those encountered in the field; the food supply was consistent, e.g. the standing crop of phytoplankton in Lake George (Burgis *et al.*, 1973; Ganf & Viner, 1973) and the feeding ecology of the fish species was well researched with more or less consistent diurnal rhythms, synchronisation of feeding and constant food selectivity.

For the feeding curves to be useful in ecological modelling they should cover both high and low algal concentrations, including the critical and threshold concentrations respectively (Frost, 1975). The behaviour of grazing at low algal densities is important as it has a strong effect on the predicted stability of the plankton (Seale, 1974). Thus, if the aim of the quantitative laboratory trials had been to help in the ecological modelling of fertilized ponds, feeding trials would need to be completed on large, colonial *Microcystis aeruginosa*. This was found to be the prominant, bloom-forming algal species in fertilized ponds in Thailand. Ingestion rates for *O. niloticus* would have to be measured over a wide range of biovolume concentrations, including feeding rates around the threshold concentration and maximum ingestion rates at high algal densities.

In summary, the various studies of this thesis have helped contribute towards a clearer picture of the filter feeding mechanism and feeding ecology of *O. niloticus*.

Despite an extensive histological and ultrastructural study of the pharyngeal apparatus, the microbranchiospines remain an enigma. Their role in filter feeding can at best only be described as incidental. However, the study has substantiated the mucus entrapment theory of Greenwood (1953), and indicated the differential role of the various mucosubstances depending on their histochemistry. Sulphated mucus is proposed to act as a 'heavy' lubricant in areas of the feeding apparatus where abrasion is prevalent. Sialyated

and neutral mucins, having a fibrous, net-like structure, are proposed to function in the actual filtering mechanism.

The development of the pharyngeal apparatus was observed to be complete at 40 mm S.L. and fish of this size were capable of ingesting suspended phytoplankton. The quantitative filter feeding curves of *O. niloticus* were similar to those of other aquatic suspension feeders, including zooplankton and anuran tadpoles. This strengthens a theory of universality in the regulatory mechanism of filter feeders.

The length at which *O. niloticus* were able to filter feed in the laboratory was less than reported in the natural habitat. The fieldwork in Thailand demonstrated that *O. niloticus* held in the same environment with identical food resources showed no ontogenetic shift in diet, at least between 35 and 80 mm S.L. Therefore, it is concluded that the feeding apparatus of *O. niloticus* allows ingestion of a varied diet. The shift in diet and feeding mode, observed in the natural habitat, from omnivorous particulate feeding to phytoplanktivorous filter feeding is probably due to a change in the food resource and does not correspond with a change in functional morphology.

Appendix 1

Water quality, feeding ecology and histological details of the pharyngeal mucus cells of *O. niloticus* from the ponds at Tinsulanonda Songkhla Fisheries College, Songkhla, S. Thailand.

(a) Pond 7 Water quality : Secci disc 20 cm
pH 4.1
salinity 7.7 %

Fish S.L. (mm)	SFI	Stomach contents (% volume occurrence)			
		Small, unicellular phytoplankton	Detritus	bacterio-plankton	Rotifera
78	3	10	80	10	-
75	3	5	90	5	+
73	4	5	80	5	10
78	3	+	100	-	-
Water Sample		20	70	10	+

Histological details : Large numbers of clavate mucus cells associated with the gill rakers. Stain purple to blue following alcain blue (2.5) / APS and bright pink to pale pink after alcian blue (1.0) / PAS, therefore containing both neutral and sialyated mucosubstances.

(b) Pond 13. Water quality : Secci disc 50 cm
pH 3.6
salinity 8.6%

Fish S.L. (mm)	SFI	Stomach contents (% volume occurrence)	
		Detritus	Copepoda (nauplii present)
			25 (+)
80	4	75	30 (+)
85	4	70	60 (+)
82	4	40	5 (-)
95	3	95	
Water sample		70	30 (+)

Histological details : Large numbers of clavate mucus cells associated with the gill rakers. Stain purple to purple/blue following alcian blue (2.5) / PAS and bright pink to pink after alcian blue (1.0) / PAS and bright pink to pink after alcian blue (1.0) / PAS, therefore containing both sialyated and neutral mucosubstances.

Appendix 2

Histological details of the pharyngeal mucus cells of *Oreochromis (Alcolapia) alcalicus grahami* (Boulenger) from Lake Magadi and Lake Nakuru, Kenya.

Water quality of Lake Magadi : (from Beadle, 1974)		
	Salinity	upto 40%
	pH	10.5
	Temperature	upto 40°c
	80% salts	Na HCO_3 & $Na_2 CO_3$
	Alkalinity	200 meq l^{-1}

(a) Source : British Museum (Natural Histroy),
Cromwell Road,
London.

Fish	Museum & Reg No	Fish S.L. (mm)	Locality
1	1967.9.13.30-39	44	Hot springs between high Magadi beds.
2	1979.3.5.366-400	45	L. Nakuru (introduced).
3	1982.4.22.32-35	80	1st evaporation basin, Hot springs, L. Magadi.

Histological details

1-3 Large, clavate mucus cells associated with the gill rakers in dense accumulations. Stain purple/blue to blue following alcian blue (2.5) / PAS and pale pink after alcian blue (1.0) / PAS, therefore containing a predominance of sialyated mucosubstances.

(b) Source : National Museum,
Box 40658,
Nairobi, Kenya.

Fish No.	Date & Reg.No.	Fish S.L. (mm)	Locality
4	20.II.77	53	Hot springs, southern end of L. Magadi.
5	20.II.77	71	Hot springs, southern end of L. Magadi.

Stomach Contents

Food item	Fish no. 4 % volume	Fish no. 5 occurence	Food item
Plant material	20	5	*Spirulina*
detritus	20	40	*Oscillatoria*
Phytoplankton (unicells)	5	35	*Nitzschia*
small fish	55	+	*Microcystis / Lyngbya*
		20	detritus
		+	copepoda

Histological details

4 Few mucus cells associated with pharyngeal apparatus, more common towards rear of chamber. Stain blue following alcain blue (2.5) / PAS and pale pink for alcian blue (1.0) / PAS, therefore, containing a predominance of acidic mucosubstances. Mucus cells of tooth sockets of pharyngeal pads stain blue for alcian blue (1.0) / PAS, therefore, a sulphated mucosubstance.

5 Large, clavate mucus cells associated with the gill rakers in dense accumulations. Staining as above, i.e. a predominance of sialyated mucus producing cells in the gill rakers and sulphated mucosubstance producing cells in the tooth sockets of the pharyngeal pads.

Appendix 3

Estimation of the ingestion rate of 35 mm S.L. *O. niloticus* during the diurnal feeding trial at A.I.T., Bangkok, Thailand.

Fish No	Rotifera	Biovolume contribution to diet (μm^3) Copepoda	Nauplii	Cladocera	Total Zooplankton
1	1.0607×10^9	2.1786×10^9	4.2311×10^7	6.7603×10^8	3.9576×10^9
2	1.9283×10^9	1.1883×10^9	1.4280×10^7	4.5069×10^8	3.5816×10^9
3	1.4945×10^9	1.7825×10^9	2.4858×10^7	5.9716×10^8	3.8990×10^9

(a) The zooplankton accounted for 30% of the stomach contents biovolume. Therefore, the total stomach contents biovolume was calculated as total zooplankton biovolume x 100/30.

(b) The feeding period was from 1200 to 1500 hours, therefore a total of 3 hours.

(c) The mean wet weight of the three fish sampled was 1.56 ± 0.16g.

Fish No	Stomach contents biovolume (μm^3)	Ingestion rate (μm^3 fish^{-1} h^{-1})	Ingestion rate (μm^3 g^{-1} h^{-1})
1	1.3192×10^{10}	4.3973×10^9	2.8188×10^9
2	1.1939×10^{10}	3.9797×10^9	2.5511×10^9
3	1.2997×10^{10}	4.3323×10^9	2.7771×10^9

The mean ingestion rate (μm^3 g^{-1} h^{-1}) = $2.7157 \pm 0.1176 \times 10^9$

Appendix 4

Published Paper

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The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*)

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(*Accepted 20 August 1987*)

(With 6 plates and 3 figures in the text)

The development, ultrastructure and histology of the buccopharyngeal filter-feeding apparatus of *Oreochromis niloticus* (Trewavas) was investigated using light and scanning electron microscopy. Gill rakers are present as small groups of taste-buds in fish of 9 mm standard length and development is complete by 40 mm standard length. Associated with the gill rakers are numerous taste-buds and mucous cells producing a sialyated mucosubstance.

Exponential relationships were found between fish standard length and gill raker number, width and spacing.

The pharyngeal jaws are toothed structures with a complex fine structure. Tooth development appears to be initiated at the base of the pads. Production of a sulphated mucus occurs from mucous cells in the tooth sockets. Development of the pads appears complete by 40 mm standard length.

The development of the buccopharyngeal filter feeding apparatus is correlated with published observations on temporal changes in diet. The function of the different types of mucus in filter feeding is discussed.

Contents

	Page
Introduction	133
Materials and methods	134
Fish	134
Preparation	134
Results	135
Morphometrics	135
Scanning electron microscopy (SEM)	136
Histology	137
Surface ultrastructure of the gill arch	137
Fine structure of the gill arch	139
Structure and development of the pharyngeal pads	141
Discussion	144
Summary	147
References	147
Abbreviations used in plates	149

Introduction

In the natural habitat, the feeding mode and diet of the tilapia *Oreochromis niloticus* (Trewavas) changes from omnivorous particulate feeding, when young fry, to phytoplanktivorous filter feeding by 60 mm total length (Trewavas, 1983). Although the phytoplanktivorous diet of *O.*

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niloticus is well documented (Fryer & Iles, 1972; Moriarty & Moriarty, 1973; Trewavas, 1983), the mechanisms by which filter feeding takes place are poorly understood.

A qualitative and quantitative assessment of the role of gill rakers in filter feeding in fishes has been made by Hyatt (1979). Many workers have considered gill rakers as passive sieves (Durbin & Durbin, 1975; Rosen & Hales, 1981; Drenner, Mummert, de Noyelles & Kettle, 1984). Whilst this method of filter feeding seems feasible in species with elaborate, comb-like rakers, such as the anchovies (*Engraulis anchiota*, *E. japonica*, *E. capensis*), pilchard (*Sardinops ocellata*), paddle fish (*Polyodon spathula*) and goldfish (*Carassius auratus*) (Dz. de Ciechomski, 1967; Shen, 1969; Iwata, 1976; King & MacLeod, 1976; Rosen & Hales, 1981), the gill raker morphology of *O. niloticus* does not appear to represent an efficient passive filtering device, as the gill rakers are comparatively small and widely spaced.

Rubenstein & Koehl (1977) state that the presence of mucus-covered elements in biological filters is evidence that aerosol feeding, in which particles are attracted to a sticky filter, occurs. However, this theory remains largely untested and to date aerosol suspension feeding has been demonstrated only in the brittle star *Ophiopholis aculeata* (La Barbera, 1978). In other animals, such as anuran and lamprey larvae, mucus is purported to play some role in food entrapment (Savage, 1952; Kenny, 1969*a*, *b*; Wassersug, 1972; Mallat, 1981), whilst in the Atlantic menhaden *Brevoortia tyrannus*, Friedland (1985) has proposed that particulate food is primarily captured by mechanical sieving, although its transport could be mucus mediated.

In tilapias, too, theories of filter feeding remain largely speculative. Greenwood (1953) has suggested that suspended phytoplankton is drawn into the buccal cavity where it becomes entrapped with copious quantities of mucus, although this has yet to be demonstrated. Speculations on the possible role of microbranchiospines, a continuous row of small, toothed, bony structures which lie at the base of the gill filaments in filter feeding, have recently been disproved (Beveridge, Briggs, Mowat, Northcott & Ross, 1987).

Posterior to the gill arches in *O. niloticus* are dorsal and ventral toothed pharyngeal pads. Ebeling (1957) proposes the use of pharyngeal teeth in the actual filtering process of eastern Pacific mullets. However, Greenwood (1953) and Fryer & Iles (1972) suggest that the function of the pads in *Oreochromis* species is simply to rake the mucus-entrapped food particles into the oesophagus.

In this paper, a detailed ultrastructural and histological study of the structure and development of organs associated with filter feeding in *O. niloticus* is presented in an attempt to clarify feeding mechanisms.

Materials and methods

Fish

Specimens of *O. niloticus* examined were derived from genetically homogeneous stock held at the Institute of Aquaculture (McAndrew & Majumdar, 1983).

Preparation

Buccopharyngeal feeding structures, the gill arches and upper and lower pharyngeal pads were carefully removed from the fish using a stereo-zoom dissecting microscope. Gill arches used for the measurement of gill raker number, width and spacing were examined fresh. Following a preliminary examination of the gill arch apparatus which revealed that apart from the outer gill rakers of gill arch I and the inner rakers of arch IV, the structure and size of the rakers was very similar, data was collected from the outer row of rakers of gill arch II.

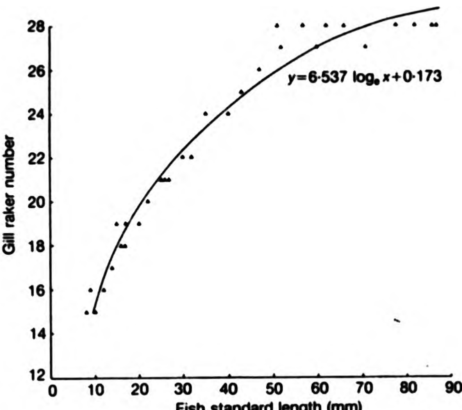

FIG. 1. The relation between gill raker number and fish standard length.

For fish of standard length (S.L.) ≤ 35 mm, the dissected gill arch was placed on a microscope slide and the rakers enumerated by counting the bony gill raker bars using a light microscope. With this method it was possible to obtain data from fish as small as 8 mm S.L. The gill rakers of fish ≥ 40 mm S.L. were counted using the stereo-zoom microscope. The gill raker widths and spacing of fish 14–68 mm S.L. were measured using a calibrated ocular graticule and light microscope. Ten measurements of the above parameters per fish were taken from the middle section of the right gill arch.

Examination of the sections showed that there were 2 distinct types of mucous cell present in the gill arches; large, clavate cells and small, superficial goblet cells. Thirty measurements for each cell type were made from each fish. Cells were measured in 6–10 sections per animal. The clavate cells were measured using the longest axis.

The density of the small mucous cells was estimated from the anterior face of the gill arch. The number of cells in a 250 μm length of epithelium was counted. Cells were counted in 8–10 sections per animal.

An accurate assessment of the density of the large mucous cells was not attempted.

Results

Morphometrics

Figure 1 shows the development of gill raker number in fish of 8–87 mm S.L. The maximum number, 28, of gill rakers on the arch is reached at 50–60 mm S.L. The relationship between fish standard length and gill raker number is ($y = 6{\cdot}537$ loge $x + 0{\cdot}173$; $r = 0{\cdot}98$).

Figure 2 shows the change in gill raker width and spacing in fish of 14–68 mm S.L. For each fish analysed, the gill rakers were wider than the inter-raker spacing. The relationships between fish

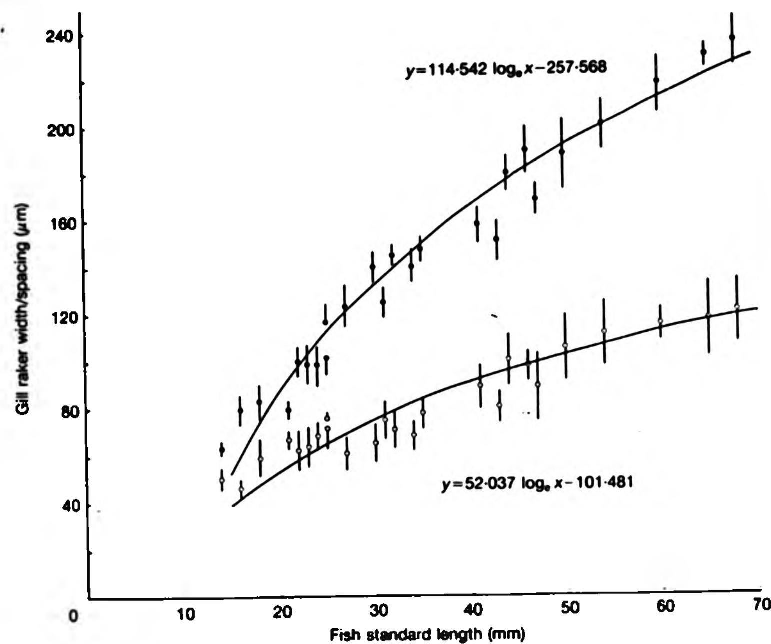

FIG. 2. The relation between gill raker width (●) and spacing (○) and fish standard length (mean ± 1 S.D.).

standard length and gill raker width ($y = 114 \cdot 542 \log_e x - 257 \cdot 568$; $r = 0 \cdot 98$) and standard length and inter-raker spacing ($y = 52 \cdot 037 \log_e x - 101 \cdot 481$; $r = 0 \cdot 94$) are also both exponential.

Scanning electron microscopy (SEM)

The ultrastructure and development of the feeding apparatus was studied using an ISI-60A scanning electron microscope. Intact pharyngeal apparatus or individual gill arches and pharyngeal jaws were rinsed briefly in distilled water before being placed in freshly prepared 2·0% paraformaldehyde and 2·5% glutaraldehyde in 0·1 M cacodylate-HCl, 2 mM calcium chloride buffer (pH 7·4). After at least 2 h fixation at 4 °C, the material was rinsed with several changes of buffer. Specimens were then fixed for at least 2 h at 4 °C in buffered 1% osmium tetroxide. They were then dehydrated through a graded acetone series, stored in 70% acetone at 4 °C if necessary, and critical-point dried in a Polaron dryer, before being mounted on brass stubs with silver conducting paint and coated with a layer of gold in an Edwards S150 sputter coater.

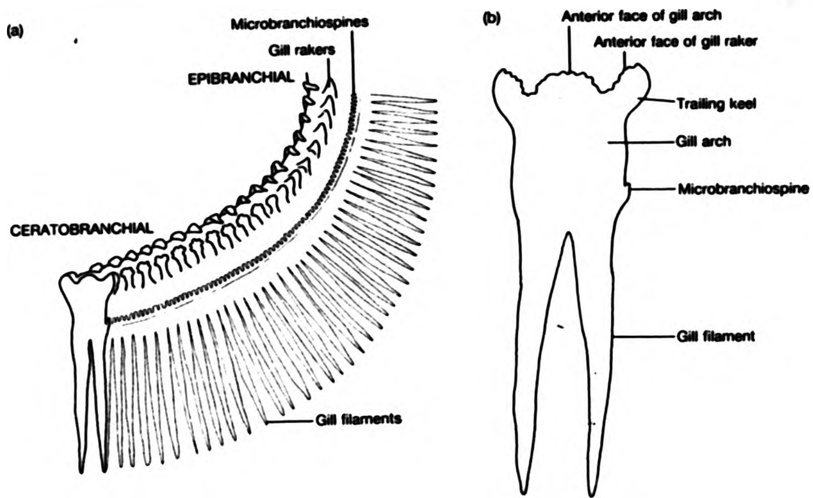

FIG. 3. (a) Diagram showing the general structure of a gill arch from the tilapia, *Oreochromis niloticus*. Gill arch from the left side of the pharyngeal 'basket'. (b) Diagram of transverse section of a gill arch.

Histology

Complete heads or dissected gill arches were fixed in 10% neutral buffered formalin, processed and embedded in wax. Longitudinal and transverse sections were made of individual gill arches. Transverse sections were taken from complete heads of fish 40 mm S.L. All sections were 5 μm in thickness.

Sections were stained with haematoxylin-eosin (HE) for structural analysis or alcian blue (pH 1·0 or 2·5)/Periodic Acid Schiff (PAS) for identification of the mucous cell glycoproteins (see Drury & Wallington, 1980, for details). All sections were examined using a light microscope.

Surface ultrastructure of the gill arch

A preliminary examination of the pharyngeal region revealed that, although there were slight differences in structure, the surface features of the four gill arches were very similar. Each gill arch supports two rows of gill filaments and two rows of gill rakers (Fig. 3a, b). Gill arch I differed from the other arches in that the outer rakers were more elongate forming the boundary with the operculum. The inner row of rakers had the same structure as those of arches II and III and the outer row of rakers of gill arch IV. Gill arch IV differed in that it was much shorter, laterally widened and the inner, elongate rakers overlapped each other with no, or very few, inter-raker spaces. The inner rakers of gill arch IV formed the boundary with the lower pharyngeal jaw.

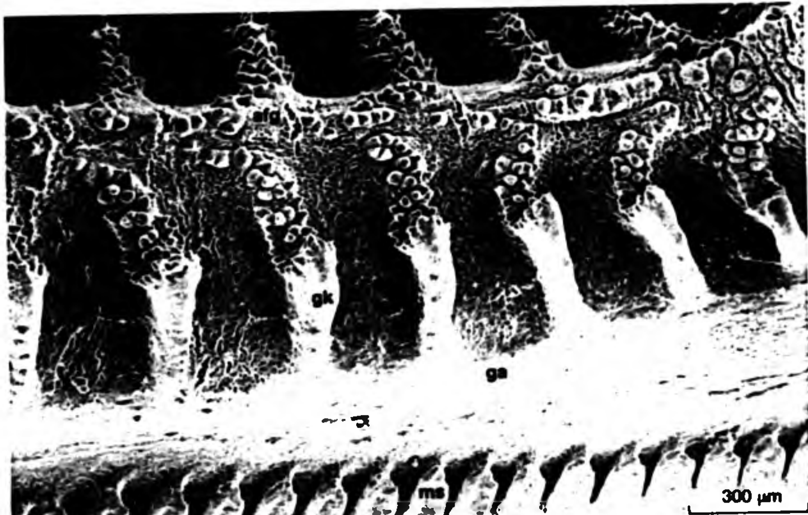

PLATE I. Scanning electron micrograph (SEM) of the second gill arch from a fish 65 mm S.L. (Explanation of abbreviations see p. 149.)

Plate I shows gill arch II from a fish of 65 mm S.L. illustrating the general features. It is apparent that material fixed as described is free of mucus. At high power it was very difficult to find mucus cell pores, which suggests that this procedure fixes them in the closed state. All surfaces of the gill arch and rakers are covered with a pavement epithelium that has concentric surface microridges. A feature of the gill arch is the presence of the 'club-like' gill rakers.

Gill arch development was studied in fish from 9 mm S.L. upwards (Plate II a–d). The gill rakers of fish 9–10 mm S.L. (Plate IIa) consist of small outgrowths composed of 2–4 taste-bud papillae. The anterior leading face of the arch is covered in rows of taste-buds that connect opposite rakers on the arch. The gill rakers of fish 14–18 mm S.L. (Plate IIb) had further developed into more prominent outgrowths bearing about 10–14 taste-buds.

The gill rakers had also started to differentiate a posterior trailing section, termed the 'keel', and an anteriorly directed leading face. The gill arch face is covered in rows of taste-buds. Plate II c and d illustrate further development of the gill rakers. The 'mature' structure of the raker is reached at about 40 mm S.L. and is shown in Plate II d. The gill raker has a smooth trailing keel that is continuous with the side of the gill arch. The two leading edges of the gill raker each carry a single row of taste buds and the face contains a multiple row that extends on to the anterior face of the gill arch. The occurrence of taste-buds in rows on the face of the gill arch decreases in fish of 40 mm S.L. upwards.

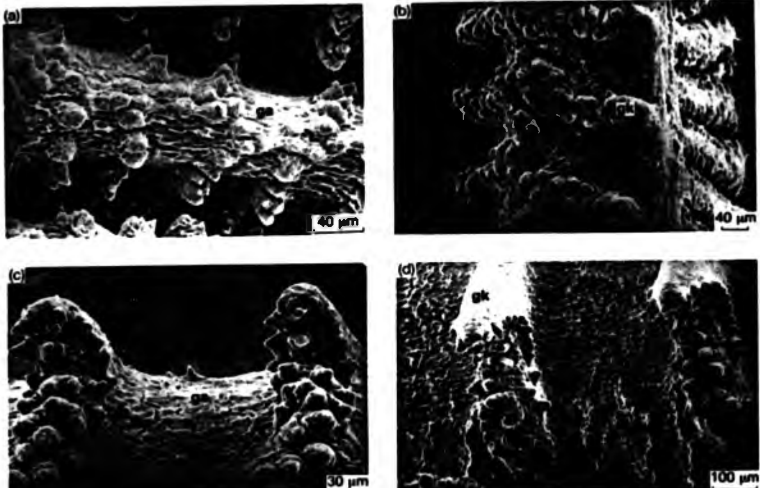

PLATE II. SEM study of gill raker development on the second gill arch. (a) 9 mm S.L. fish; (b) 16 mm S.L. fish; (c) 40 mm S.L. fish; (d) 100 mm S.L. fish. (Explanation of abbreviations see p. 149.)

Fine structure of the gill arch

The staining of sections with alcian blue (pH 1·0 or 2·5)/PAS revealed the presence of two distinct types of mucous cell associated with the gill arches; small, superficial cells that were typically goblet-shaped and large, deeper penetrating cells that were clavate. The small mucous cells stained either red or a combination of red/blue following alcian blue (pH 2·5)/PAS treatment and contained either neutral or a mixture of neutral/acidic glycoproteins, respectively. They stained red after treatment with alcian blue (pH 1·0)/PAS. The large, clavate mucous cells stained blue following alcian blue (pH 2·5)/PAS and faint pink following alcian blue (pH 1·0)/PAS indicating a major predominance of acidic mucosubstances. No mucous cells stained blue after alcian blue (pH 1·0)/PAS. Therefore there were no sulphated mucosubstances associated with gill arch or rakers.

The gill rakers are covered with a compact squamous epithelium that overlies a stratified epithelium of 35 to 60 μm thickness in fish of 40–200 mm S.L. A transverse section of a gill raker, Plate III, shows that the epithelium of the anterior face contains many taste-buds as observed using SEM, and a few mucous cells. A detailed examination of the material revealed that the large mucous cells occurred only in association with the trailing keel of the gill raker, Plate III. The epithelium of the keel also contained the small type mucous cell in reduced numbers. The thick, stratified epithelium of the gill raker stands on a basement membrane of about 2·5 μm thickness. The centre of the raker is filled with a loose, vascularized connective tissue and contains a single

PLATE III. (a) Transverse section of gill arch from a 61 mm S.L. fish alcian blue/PAS × 200. (b) Transverse section of gill arch from a 22 mm S.L. fish alcian blue/PAS × 400. (c) Transverse section of gill raker from a 78 mm S.L. fish alcian blue/PAS × 200. (Explanation of abbreviations see p. 149.)

bony gill raker element, observed in whole mounts of fish from 8 mm S.L. upwards. The epithelium covering the anterior face and sides of the gill arch is considerably thinner, 8–12 μm, and contains only small type mucous cells and few taste-bud papillae.

A study of gill arch development as observed from histological preparations revealed several interesting features. The mucous cells of fish of about 14 mm S.L. were the small, goblet type, and there were no large clavate cells associated with the gill raker buds. The large mucous cells first became apparent in fish of 18 mm S.L. However, in fish 18–22 mm S.L. the number of acidic cells associated with the gill rakers was low and only occurred towards the posterior of the feeding chamber. In fish of 24 mm S.L. there is an increase in the number of clavate mucous cells associated with the keel of the gill raker and an increase in the size (Table I). At this time there is also an increase in the general occurrence of the large mucous cells so that by about 36 mm S.L. they occur in all gill rakers on all gill arches.

TABLE I

Relation of mucous cell dimensions (μm) and goblet mucous cell concentration on the gill arch to fish standard length (mm). Figures in parenthesis are ± 1 S.D.

Fish standard length (mm)	Mucous cell dimensions (μm)		Goblet cell concentration (cells) 250 μm epithelium
	Clavate	Goblet	
14	—	8·93(1·01)	11·50(2·46)
14	—	9·10(1·23)	—
18	15·97(1·36)	9·23(1·59)	13·90(2·51)
22	16·30(1·83)	9·89(1·30)	14·50(2·92)
24	19·43(3·28)	9·97(1·42)	—
26	19·60(3·30)	10·47(1·29)	16·70(3·40)
33	23·42(2·14)	10·64(1·20)	—
36	28·49(2·36)	10·55(1·44)	16·90(2·47)
41	26·10(2·89)	11·13(1·45)	—
62	30·38(4·17)	12·03(1·65)	16·88(3·68)
77	31·45(3·21)	12·44(2·12)	17·13(3·36)
103	31·54(3·04)	12·03(1·25)	16·90(3·45)
168	33·88(4·25)	11·95(1·42)	17·30(3·95)

A quantitative estimation of large mucous cells in the keel of the gill rakers was not attempted. However, the increase in number with increasing fish size is apparent from Plate III a–c.

Table I shows the relationship of various mucous cell parameters to fish standard length. There is a noticeable increase in size of the clavate mucous cells, although there is only a slight increase in the diameter of the goblet mucous cells with increasing fish standard length. There was a significant difference ($P < 0·001$) between the dimensions of large clavate and small goblet cells in all fish examined. The concentration of goblet mucous cells on the gill arch increases in fish up to about 26 mm S.L. There were significant differences ($P < 0·001$) between the mucous cell concentration of fish of 14 mm S.L. and fishes of 26 to 168 mm S.L. ($t = 3·91$; $d.f. = 18$; $P < 0·05$) and between fishes of 18 and 168 mm S.L. ($t = 2·30$; $d.f. = 18$; $P < 0·05$).

Structure and development of the pharyngeal pads

Although histological sections were made of both upper and lower pharyngeal jaws, a detailed description is not given as previous work on *Astatotilapia elegans* (Ismail, Verraes & Huysseune, 1982 and Huysseune, 1983) deals with the fine structure and process of tooth development of these structures.

Plates IV a–c and V a–c show the development of upper and lower pharyngeal jaws, respectively. It can be seen that there is a proliferation in the number of teeth between 14 and 24 mm S.L. Development of the pharyngeal pads with respect to shape and tooth density appears complete at 40 mm S.L.

The epithelium between the teeth has surface microridges and on the lower pharyngeal jaws of fish about 14 mm S.L. there were many taste-buds. These taste-buds were not borne on papillae and did not, therefore, project far above the level of the neighbouring epithelial cells (Plate VI). The occurrence of taste-buds on the jaws decreased in fish of 14 mm S.L. upwards as the number of teeth increased.

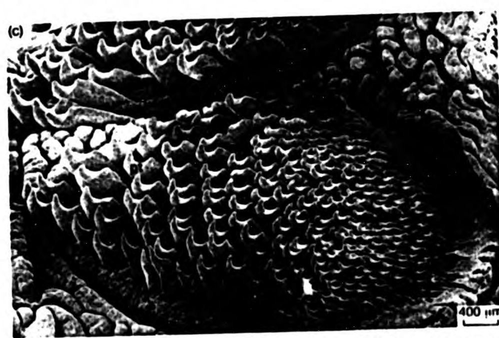

PLATE IV. SEM study of upper pharyngeal pad development. (a) 14 mm S.L. fish; (b) 24 mm S.L. fish; (c) 57 mm S.L. fish. (Explanation of abbreviations see p. 149.)

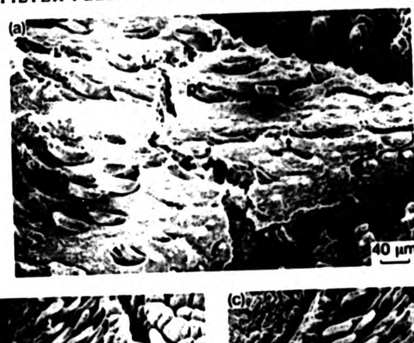

PLATE V. SEM study of lower pharyngeal pad development. (a) 14 mm S.L. fish; (b) 24 mm S.L. fish; (c) 40 mm S.L. fish.

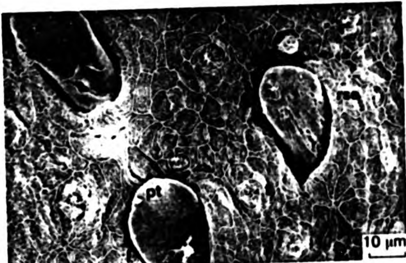

PLATE VI. Close up of lower pharyngeal pad from a 14 mm S.L. fish.

The teeth project above the stratified squamous surface epithelium of the pharyngeal pads via tooth sockets that are lined with large spherical mucous cells. The diameter of the cells was $14{\cdot}99 \pm 1{\cdot}22\ \mu m$ in a fish 36 mm S.L. These cells stain blue following alcian blue (pH 1·0)/PAS and, therefore, contain a sulphated glycoprotein. The mucous cells of the pharyngeal jaws were less common in fish 14–22 mm S.L. but were numerous in fish of 36 mm S.L. During histological sectioning and staining of whole heads, it was noted that there were sulphated mucus-producing cells lining the sockets of the mandibular teeth.

The brown coloured caps of the pharyngeal teeth, visible in fresh material, were absent following decalcification.

Discussion

This study revealed the surface and histological features of the pharyngeal structures associated with filter feeding in *O. niloticus* of 9 mm up to 168 mm S.L., covering both the particulate and filter feeding periods of their life histories.

Gill rakers were observed as small projections of taste-buds on the gill arches of fish 9 mm S.L., gill raker number reaching a constant value at 50–60 mm S.L. Such aspects of gill raker development in planktivorous fishes have been described by various authors Dz. de Ciechomski (1967), Shen (1969), Iwata (1976), King & MacLeod (1976), O'Connell (1981) and Rosen & Hales (1981).

Most studies on the filter feeding mechanism in fish have assumed a method of mechanical sieving by the gill rakers. Measurement of gill raker length and inter-raker distance in determining mesh size has been performed by King & MacLeod (1976), Durbin (1979), Wright, O'Brien & Luecke (1983). However, King & MacLeod (1976) point out that a filtering mesh, finer than measurements suggest, result from interlocking of the gill rakers and their denticles on the upper and lower arches. The construction of a fine mesh due to denticles on the gill rakers has been noted by Dz. de Ciechomski (1967) and Shen (1969). Iwata (1976) described the fusion of parts of adjacent gill rakers in the phytoplanktivorous silver carp, *Hypophthalmichthys molitrix*.

The short, club-like gill rakers of *O. niloticus* are not typical for a planktivorous mode of feeding whereby mechanical sieving is the only means of filtration. The shortness of the gill rakers and their relatively wide spacing would apparently not produce an effective filter. However, observation of fresh specimens revealed that the gill rakers of neighbouring arches do interdigitate. Although results show the gill rakers to be wider than the inter-raker distances, interdigitation probably occurs due to the structures being fleshy protuberances filled with much loose connective tissue. The interdigitation of gill rakers has been observed in the phytoplanktivorous *Tilapia esculenta* (Greenwood, 1953). From histological sections of whole heads and general dissection, it was apparent that the buccal cavity is dorsoventrally flattened. Liem & Osse (1975) state that such a cavity can be more easily enlarged by movements of the floor of the mouth, whereas a mouth with greater depth may benefit from lateral movement of the side walls. The passage of water through the buccal cavity of *O. niloticus*, due mainly to movements of the mouth floor, would not require excessive lateral movement of the gill arches. This, combined with the tightness of the interdigitation due to wider gill rakers than inter-raker distance, may serve to limit the extent of lateral movement of the gill arches maintaining the 'sieve'.

In live fish, the gill arches are orientated so that the anterior face and those of the gill rakers are exposed to the water current. The trailing keel of the gill rakers abut adjacent gill rakers of

neighbouring gill arches and perhaps the neighbouring arch itself. In this alignment the taste-buds of the gill rakers face into the water current, whereas the large mucous cells of the trailing keel face into the pores of the sieve. Iwai (1964) gave a comprehensive account of taste-bud occurrence in freshwater, marine and euryhaline species. In fishes with numerous taste-buds in the branchial region, they are more densely arranged along the anterior surface of the gill rakers and arches so that they are near the pathway of engulfed food and respiratory currents. This was observed to be the case in *O. niloticus*. Iwai states that if the gill rakers play an essential part in a filter feeding mechanism, a sensory function for concentrating materials on the gill rakers is expected. The association of taste-buds with the gill arches and rakers of *O. niloticus* from early in development (9 mm S.L.) suggests the importance of these structures in filter-feeding.

Reutter, Breipohl & Bijvank (1974) described three types of taste-buds in the head gut of *Xiphophorus hekleri* Heckel. Due to the depression around the base of some taste-buds, it was hypothesized that they may also serve a mechanoreceptor function. The rows of taste-buds on the anterior edges of the gill rakers may also serve such a function due to their isolation in a single projecting row. The taste-buds of the gill rakers of *O. niloticus* were clearly larger and more numerous than those occurring on the vela, tongue and palate and this is the opposite of what has been found in *X. helleri*. These differences may be related to feeding habit, as *X. helleri* is an omnivorous particulate feeder and, therefore, presumably oral and buccal handling of food items is more important than processing by the gill rakers.

A detailed account of the structure and distribution of taste-buds in the oro-pharyngeal wall of *Cyprinus carpio* is given by Sibbing & Uribe (1985).

Mucous cells were not common in the thick, stratified, squamous epithelium of the gill rakers and arches that contained the taste-buds. However, Reutter (1980) suggests that in fish the large receptor villi of the taste-bud chemosensory cells possess only a very thin mucous surface coat, if any, and that they seem to contact taste molecules directly. The perception of certain amino acids as chemical feeding stimulants has been shown for the herbivorous fish *Tilapia zillii* by Johnsen & Adams (1986).

The anterior face and sides of the gill arch contain small, goblet mucous cells, whereas the keel of the gill rakers is characterized by large, clavate mucous cells. Two distinct types of mucous cell have been shown by Asakawa (1970), Yamada & Yokote (1975) and Bullock, Roberts & Gordon (1976), although their morphological differences were not associated with differences in histochemical nature. Saxena & Kulshrestha (1981) found two well differentiated mucous cells that secrete different mucosubstances in the epidermis of *Mystus (mystus) vittatus*.

The elongate mucous cells associated with the trailing keel of the gill rakers secrete an acidic mucosubstance. In mammalian systems, viscosity increases as the acidic glycoprotein content of the mucus increases (Jones, Bulduc & Reid, 1973; Iravani & Melville, 1974), and as the composition of mucus from fish and mammals is similar, its viscosity probably changes in a similar manner (Solanki & Benjamin, 1982). Due to the occurrence and type of mucus produced by the clavate mucus cells, it is proposed that this mucus forms a 'net' in the pores of the branchial sieve. This mucus may also have increased particle capturing properties due to its charged acidic groups. The function of charge in the capture of particles by the Pacific brittle star has been shown by LaBarbera (1978).

Flood (1981) found a net-like ultrastructure for the feeding mucus of various marine animals and stated that it could be a fundamental property of mucus. This property would enhance the ability of mucus secreted by the gill rakers to act as an actual sieve.

The presence of the neutral and neutral/acidic mucus on the anterior face and medial and lateral sides of the gill arch may act in regulating transport of the captured particles to the posterior of the buccopharyngeal chamber. It is assumed that this mucus would be less viscous.

The only differential distribution of mucus cells on the gill arches of *O. niloticus* was the association of the large clavate cells with the trailing keel of the gill rakers. There were no apparent differences between sections of the same gill arch or between different gill arches. Friedland (1985) showed that there were higher proportions of acidic cells on gill arch IV in the Atlantic menhaden, and suggests that, together with the spacing of branchiospinules on the gill rakers, the gill arches become progressively better at filtering small particles from exterior to interior. Furthermore, it was shown that there was a differential distribution of mucous cells on the gill rakers. Mallat (1981) found that in the pharynx of the larval lamprey alcian blue non-reactive cells were located lateral to alcian reactive cells, in a situation where only small, easily trapped particles were likely to reach.

Sibbing & Uribe (1985) found that mucous cells producing sialomucines occurred in the anterior part of the oro-pharynx of the carp and that sulfomucines only appeared in the posterior part of the pharynx. These mucosubstances were produced by morphologically different types of mucous cells. It was hypothesized that the highly viscous sulfomucines aid in trapping small particles, aggregating them into boluses, and lubrication of the chewing pad, and that the less viscous sialomucines maintain a laminar flow during suction and lubrication of particle handling in the pharynx.

The use of mucus in the feeding mechanisms of anuran larvae has been reported by many workers including Savage (1952), Kenny (1969*a*, *b*) and Wassersug (1972). Kenny (1969*b*) found the mucus-secreting epithelium of various species to be PAS positive but alcian blue non-reactive, and stated that it contained no protein, being a glycolipid. However, its viscosity could still be high as Law (1960) states that the 'cord factor' of tuberculosis bacilli, associated with the characteristic tendency of these organisms to aggregate in long cords, is a glycolipid. Lewis (1970) suggests that the proportion of phospholipids may determine the relative viscosity of fish mucus.

A survey of the teeth of *Oreochromis mossambicus* (Peters) has been undertaken by Lanzing & Higginbotham (1976). Trewavas (1983) describes the pharyngeal jaws of many tilapiine species and Greenwood (1953) of many *Haplochromis* species.

The taste-buds on the pharyngeal jaws are similar to those of the toothed, masticating apparatus of *X. helleri* described by Reutter *et al.* (1974). The abundance in larvae of 14–20 mm S.L., and decrease as the standard length increases, appears to be correlated with the change in diet from particulate to filter feeding (Trewavas, 1983) and the consequent change in function of the pharyngeal pads from handling single prey items to mucus-bound phytoplankton. Selection of food items may take place at the pharyngeal pads during particulate feeding on single prey items.

A distinctive feature of the mucous cells of the lips and pharyngeal pads was the production of a sulphated mucosubstance. A secretion of sulphated mucus would act as a viscous lubricant and the attachment of this lubricant to the pads would be enhanced by the microridges of the surface epithelium. The increase in mucous cell number observed in larger fish may indicate an increased need for lubrication as the diet changes to phytoplanktivory and the pads function as mechanical rakers of mucus and trapped algae into the oesophagus.

The disappearance of the brown coloured caps of the teeth following decalcification suggests the presence of enameloid. Schmidt (1969) notes that the colour is due to iron oxide associated with the enameloid. Motta (1986) suggests that his iron layer represents a specialization to harden the teeth.

resisting abrasion and cracking. The presence of a hard layer over the pharyngeal jaw teeth of *O. niloticus* would help prevent tooth damage during the raking action of the pharyngeal jaws.

Summary

A study of the structure and development of the feeding apparatus of *Oreochromis niloticus* (Trewavas) was made using light and scanning electron microscopy. The gill rakers were observed to be club-like projections of the gill arch with an anterior face covered in taste-buds and a posterior trailing keel. Development of gill raker structure was complete at about 40 mm S.L.

There were two morphologically distinct types of mucous cell associated with the branchial apparatus. The gill rakers contained large numbers of clavate mucous cells that produced a sialyated glycoprotein. It is proposed that this mucus is used as an entrapment mechanism in the pores of the branchial sieve. The clavate mucous cells first became apparent at 18–22 mm S.L., and by 36 mm S.L. they are widely distributed in the gill rakers. The mucous cells of the gill arch are small goblet cells producing a neutral or neutral/acidic mucus.

Regression analysis of the relationships between fish standard length and gill raker number, width and spacing showed that these parameters were related exponentially over the fish lengths analysed.

The study of the upper and lower pharyngeal jaws revealed aspects of surface ultrastructure and development. The pharyngeal jaws contain large mucous cells that secrete a sulphated mucus which is proposed to act as a 'heavy' lubricant during the raking action described by Greenwood (1953) and Aerts, de Vree & Vandewalle (1986). Sulphated mucous cells were also observed in the sockets of the mandibular teeth. Development of the pharyngeal pads with respect to structure and tooth density is complete by 40 mm S.L.

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REFERENCES

Aerts, P., de Vree, F. & Vandewalle, P. (1986). Pharyngeal jaw movements in *Oreochromis niloticus* (Teleostei: Cichlidae): preliminary results of a cineradiographic analysis. *Annls Soc. r. zool. Belg.* **116**: 75–82.

Asakawa, M. (1970). Histochemical studies of the mucus of the epidermis of the eel, *Anguilla japonica*. *Bull. Jap. Soc. scient. Fish.* **36**: 83–87.

Beveridge, M. C. M., Briggs, M. R. P., Mowat, A., Northcott, M. E. & Ross, L. G. (1987). The function of microbranchiospines in tilapias (Cichlidae: Tilapiini). *Proc. int. Symp. Tilapias in Aquaculture* No. 2, Bangkok, Thailand, March 16–19, 1987. Manila: ICLARM.

Bullock, A. M., Roberts, R. J. & Gordon, J. D. M. (1976). A study of the structure of the whiting integument (*Merlangius merlangus* L.). *J. mar. Biol. Ass. U.K.* **56**: 213–226.

Dz. de Ciechomski, J. (1967). Investigations of food and feeding habits of larvae and juveniles of the Argentine anchovy *Engraulis anchoita*. *Rep. Calif. coop. oceanic Fish. Invest.* **11**: 72–81.

Drenner, R. W., Mummert, J. R., de Noyelles, F. & Kettle, D. (1984). Selective particle ingestion by a filter feeding fish and its impact on phytoplankton community structure. *Limnol. Oceanogr.* **29** (5): 941–948.

Drury, R. A. B. & Wallington, E. A. (1980). *Carleton's histological technique*. 5th edn. Oxford: Oxford University Press.

Durbin, A. G. & Durbin, E. G. (1975). Grazing rates of the Atlantic menhaden *Brevoortia tyrannus* as a function of particle size and concentration. *Mar. Biol., Berlin* **33**: 265–277.

Durbin, A. G. (1979). Food selection by plankton feeding fishes. In *Predator–prey systems in fisheries management*: 203–218. Stroud, R.H. & Clepper, H. (Eds). Washington: Sport Fish. Inst.

Ebeling, A. W. (1957). The dentition of Eastern Pacific mullets, with special reference to adaptation and taxonomy. *Copeia* 1957: 173–185.

Flood, P. R. (1981). On the ultrastructure of mucus. *Biochem. Res.* 2: 49–53.

Friedland, K. D. (1985). Functional morphology of the branchial basket structures associated with feeding in the Atlantic menhaden, *Brevoortia tyrannus* (Pisces: Clupeidae). *Copeia* 1985 (4): 1018–1027.

Fryer, G. & Iles, T. D. (1972). *The cichlid fishes of the great lakes of Africa: Their biology and evolution*. Edinburgh: Oliver & Boyd.

Greenwood, P. H. (1953). Feeding mechanism of the cichlid fish *Tilapia esculenta* Graham. *Nature, Lond.* 172: 207–208.

Huysseune, A. (1983). Observations on tooth development and implantation in the upper pharyngeal jaws in *Astatotilapia elegans* (Teleostei, Cichlidae). *J. Morph.* 175: 217–234.

Hyatt, K. D. (1979). Feeding strategy. In *Fish physiology VIII. Bioenergetics and growth*: 71–113. Hoar, W. S., Randall, D. J. & Brett, J. R. (Eds). London: Academic Press.

Iravani, J. & Melville, G. N. (1974). Long-term effect of cigarette smoke on mucociliary function in animals. *Respiration* 31: 358–366.

Ismail, M. H., Verraes, W. & Huysseune, A. (1982). Developmental aspects of the pharyngeal jaws in *Astatotilapia elegans* (Trewavas, 1933) (Teleostei: Cichlidae). *Neth. J. Zool.* 32: 513–543.

Iwai, T. (1964). A comparative study of the taste buds in gill rakers and gill arches of Teleostean fishes. *Bull. Misaki Mar. Biol. Inst.* 7: 19–34.

Iwata, K. (1976). Morphological and physiological studies on the phytoplankton feeders in cyprinid fishes. I. Developmental changes of feeding organs and ingestion rates in Kawachibuna (*Carassius auratus cuvieri*), silver carp (*Hypophthalmichthys molitrix*) and nigorobuna (*C. auratus grandoculis*). *Jap. J. Limnol.* 37 (4): 135–147.

Johnsen, P. B. & Adams, M. A. (1986). Chemical feeding stimulants for the herbivorous fish, *Tilapia zillii*. *Comp. Biochem. Physiol. (A)* 83: 109–112.

Jones, R., Bulduc, P. & Reid, L. (1973). Goblet cell glycoprotein and tracheal gland hypertrophy in rat airways: the effect of tobacco smoke with or without the anti-inflammatory agent phenylmethyloxadiazole. *Br. J. exp. Path.* 54: 229–239.

Kenny, J. S. (1969*a*). Feeding mechanisms in anuran larvae. *J. Zool., Lond.* 157: 25–246.

Kenny, J. S. (1969*b*). Pharyngeal mucus secreting epithelia of anuran larvae. *Acta zool., Stockh.* 50: 143–153.

King, D. P. F. & MacLeod, P. R. (1976). Comparison of the food and the filtering mechanism of pilchard *Sardinops ocellata* and anchovy *Engraulis capensis* off south west Africa, 1971–1972. *Invest. Rep. Sea Fish. Brch Repub. S. Afr.* No. 111: 1–29.

LaBarbera, M. (1978). Particle capture by a Pacific brittle star: experimental test of the aerosol suspension feeding model. *Science, N.Y.* 201: 1147–1149.

Lanzig, W. J. R. & Higginbotham, D. R. (1976). Scanning microscopy of pharyngeal and oral teeth of the teleost *Tilapia mossambica* (Peters). *Hydrobiologia* 48 (2): 137–139.

Law, J. H. (1960). Glycolipids. *Ann. Rev. Biochem.* 29: 131–150.

Lewis, R. W. (1970). Fish cutaneous mucus: a new source of skin surface lipid. *Lipids* 5: 947–949.

Liem, K. F. & Osse, J. W. M. (1975). Biological versatility, evolution, and food resource exploitation in African cichlid fishes. *Am. Zool.* 15: 427–454.

Mallat, J. (1981). The suspension feeding mechanism of the larval lamprey *Petromyzon marinus*. *J. Zool., Lond.* 194: 103–142.

McAndrew, B. J. & Majumdar, K. C. (1983). Tilapia stock identification using electrophoretic markers. *Aquaculture* 30: 249–261.

Moriarty, C. M. & Moriarty, D. J. W. (1973). Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. *J. Zool., Lond.* 171: 15–23.

Motta, P. J. (1986). A quantitative analysis of ferric iron in butterflyfish teeth (Chaetodontidae, Perciformes) and the relationship to feeding ecology. *Can. J. Zool.* 65: 106–112.

O'Connell, C. P. (1981). Development of organ systems in the northern anchovy, *Engraulis mordax*, and other teleosts. *Am. Zool.* 21: 429–446.

Reutter, K., Breipohl, W. & Bijvank, G. J. (1974). Taste bud type in fishes. 2. Scanning electron microscopical investigations on *Xiphophorus helleri* Heckel (Poeciliidae, Cyprinodontiformes, Teleostei). *Cell Tissue Res.* 153: 151–165.

Reutter, K. (1980). SEM-study of the mucus layer on the receptor field of fish taste buds. In *Olfaction and taste* VII. Van der Starre, H. (Ed.). London: IRL Press Ltd.

Rosen, R. A. & Hales, D. C. (1981). Feeding of Paddlefish, *Polyodon spathula*. *Copeia* **1981**(2) 441-455.
Rubenstein, D. I.,& Koehl, M. A. R. (1977). The mechanisms of filter feeding: some theoretical considerations. *Am. Nat.* **111**: 981-994.
Savage, R. M. (1952). Ecological, physiological and anatomical observations on some species of anuran tadpoles. *Proc. zool. Soc. Lond.* **122**: 467-514.
Saxena, M. & Kulshrestha, S. K. (1981). Histochemical studies of mucosubstances in the epidermis of a non scaly teleost *Mystus (mystus) vittatus* Bl. (Pisces—Bagridae). *Histochemistry* **72**: 155-160.
Schmidt, W. J. (1969). Die Schmelznatur des eisenoxydhaltigen Kappe auf Teleostier-Zähnen. *Z. Zellforsch. mikrosk. Anat.* **93**: 447-450.
Shen, S.-C. (1969). Comparative study of the gill structure and feeding habits of the anchovy *Engraulis japonica* (Hout). *Bull. Inst. Zool. Acad. Sinica* **8**: 21-35.
Sibbing, F. A. & Uribe, R. (1985). Regional specializations in the oro-pharyngeal wall and food processing in the carp (*Cyprinus carpio* L.). *Neth. J. Zool.* **35**: 377-422.
Solanki, T. G. & Benjamin, M. (1982). Changes in the mucous cells of the gills, buccal cavity and epidermis of the nine-spined stickleback, *Pungitius pungitius* L., induced by transferring the fish to sea water. *J. Fish Biol.* **21**: 563-575.
Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. *Publs Br. Mus. nat. Hist.* No. 878: 1-583.
Wassersug, R. (1972). The mechanism of ultraplanktonic entrapment in anuran larvae. *J. Morph.* **137**: 279-288.
Wright, D. J., O'Brien, W. J. & Luecke, C. (1983). A new estimate of zooplankton retention by gill rakers and its ecological significance. *Trans. Am. Fish. Soc.* **112**: 638-646.
Yamada, K. & Yokote, M. (1975). Morphometrical analysis of mucosubstances in some epithelial tissues of the eel (*Anguilla japonica*). *Histochemistry* **43**: 161-172.

Abbreviations used in plates

afg	anterior face of gill arch
cmc	clavate mucous cell
ga	gill arch
gb	gill raker bud
gf	gill raker face
gk	gill raker keel
gmc	goblet mucous cell
lc	loose connective tissue
ms	microbranchiospine
pt	pharyngeal tooth
rse	ridged surface epithelium
ste	stratified epithelium
t	taste-bud

References

Erratum

Greenwood, P.H. (1981). *The Haplochromine Fishes of the East African Lakes*.
München : Kraus International Publications.

References

Aerts, P., de Vree, F. & Vandewalle, P. (1986). Pharyngeal jaw movements in *Oreochromis niloticus* (Teleostei:Cichlidae) : preliminary results of a cineradiographic analysis. *Annls. Soc. r. zool. Belg.* **116** : 75-82.

Allen, M.M. (1968). Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.* **4** : 1-3.

APHA (1985). *Standard methods for the examination of water and wastewater.* 16th edn, Washington, D.C. American Public Health Association.

Asakawa, M. (1970). Histochemical studies of the mucus of the epidermis of the eel, *Anguilla japonica. Bull. Jap. Soc. scient. Fish.* **36** : 83-87.

Balarin, J.D. & Hatton, J.P. (1979). *Tilapia : A guide to their biology and culture in Africa.* Stirling : University of Stirling Press.

Beadle, L.C. (1974). *The Inland Waters of tropical Africa : an introduction to tropical limnology.* London : Longman.

Bellingher, E. (1974). A note on the use of algal sizes in estimates of population standing crops. *Br. Phycol. J.* **9** : 157-161.

Berg, J. (1979). Discussion of methods of investigating the food of fishes with reference to a preliminary study of the prey of *Gobiusculus Flavescens. Mar. Biol.* **50** : 263-273.

Berman, T. & Pollingher, U. (1974). Annual and seasonal variations of phytoplankton, chlorophyll and photosynthesis in Lake Kinnert. *Limnol. Oceanogr.* 19 (1) : 31-54.

Beveridge, M.C.M., Briggs, M.R.P., Mowat, A., Northcott, M.E. & Ross, L.G. (1987). The function of microbranchiospines in tilapias (Cichlidae : Tilapiini). *Proc. int. Symp. Tilapias in Aquaculture* No 2, Bangkok,Thialand, March 16-19, 1987. Manila : ICLARM.

Beveridge, M.C.M., Briggs, M.R.P., Northcott, M.E. & Ross, L.G. The occurrence, structure and development of microbranchiospines among the tilapias (Cichlidae : Tilapiini). *Can. J. Zool.* (in press).

Blanc-Livini, N. & Abraham, M. (1970). The influence of environmental salinity on the prolactin - and gonadotropin - secreting regions in the pituitary of *Mugil* (Teleostei). *Gen. Comp. Endocrinol.* 14 : 184-197.

Bone, Q & Marshall, N.B. (1982). *Biology of Fishes - Tertiary Level Biology.* Glasgow : Blackie & Sons Ltd.

Bowen, S.H. (1979). A nutritional constraint in detritivory by fishes : The stunted population of *Sarotherodon mossambicus* in Lake Sibaya, South Africa. *Ecol. Morog.* 49 : 17-31.

Bowen, S.H. (1982). Feeding, Digestion and Growth - Qualitative Considerations. In *The biology and culture of tilapias.* ICLARM Conference Proceedings 7 : 141-156. R.S.V. Pullin & R.H. Lowe-McConnell (eds.). International Center for Living Aquatic Resources Management, Manila, Philippines.

Braimah, S.A. (1987). Mechanisms of filter feeding in immature *Simulium bivattatum* Malloch (Diptera : Simuliidae) and *Isonychia campestris* McDunnough (Ephemeroptera : Oligoneuriidae). *Can. J. Zool.* 65 (3) : 504-513.

Briggs, M.R.P. (1985). The structure and function of tilapiine microbranchiospines with special reference to filter-feeding. MSc Thesis. University of Stirling.

Bulloch, A.M., Roberts, R.J. & Gordon, J.D.M. (1976). A study of the structure of the whiting integument (*Merlangius merlangus* L.). *J. mar. Biol. Ass. U.K.* 56 : 213-226.

Burgis, M.J., Darlington, J.P.E.C., Dunn, I.G., Ganf, G.G., Gwahaba, J.J. & McGowan, L.M. (1973). The biomass and distribution of organisms in Lake George, Uganda. *Proc. R. Soc. Lond. B.* 184 : 271 - 298.

Dz. de Ciechomski, J. (1967). Investigations of food and feeding habits of larvae and juveniles of the Argentine anchovy *Engraulis anchoita.*. *Rep. Calif. coop. oceanic Fish. Invest.* 11 : 72-81.

Coche, A.G. (1982). Cage culture of tilapias. In *The biology and culture of tilapias.* ICLARM Conference Proceedings 7 : 205-246. R.S.V. Pullin & R.H. Lowe-McConnell (eds.). International Center for Living Aquatic Resources Management, Manila, Philippines.

Colman, J. & Edwards, P. (1987). Feeding pathways and environmental constraints in waste-fed aquaculture : balance and optimization. In *Detritus and microbial ecology in aquaculture.* ICLARM Conference Proceedings 14 : 240-281. D.J.W. Moriarty and R.S.V. Pullin (eds.). International Center for Living Aquatic Resources Management, Manila, Philippines.

Conover, R.J. & Mayzaud, P. (1984). Utilization of phytoplankton by zooplankton during the spring bloom in a Nova Scotia inlet. *Can. J. Fish. Aquat. Sci.* **41** : 232-244.

Costerton, J.W., Ingram, J.M. & Cheng, K.J. (1974). Structure and function of the cell envelope of gram-negative bacteria. *Bacteriol. Rev.* **38** (1) : 87-110.

Crowley, P.H. (1973). Filtering rate inhibition of *Daphnia pulex* in wintergreen lake water. *Limnol. Oceanogr.* **18** (3) : 394-402.

Darnell, R.M. (1958). Food habits of fishes and larger invertebrates of Lake Pontchartrain, Louisiana, an estuarine community. *Publ. Inst. mar. Sci. Univ. Tex.* **5** : 353-416.

Darnell, R.M. (1964). Organic detritus in relation to secondary production in aquatic communities. *Verh. int. Verein. theor. angew. Verein. Limnol.* **XV** : 462-470.

Datta Munshi, J.S., Ojha, J., Ghosh, T.K., Roy, P.K. & Mishra, A.K. (1984). Scanning electron microscopic observations on the structure of gill-rakers of some freshwater teleostean fishes. *Proc. Indian natn. Sci. Acad.* **B50** (6) : 549-554.

Deason, E.E. (1980). Potential effect of phytoplankton colony breakage on the calculation of zooplankton filtration rates. *Mar. Biol.* **57** : 279-286.

De Silva, S.S., Perera, M.K. & Maitipe, P. (1984). The composition, nutritional status and digestibility of the diets of *Sarotherodon mossambicus* from nine man-made lakes in Sri Lanka. *Env. Biol. Fish.* **11** (3) : 205-219.

Dokulil, M. (1983). Aspects of gut passage of algal cells in *Sarotherodon mossambicus* (Peters), (Pisces, cichlide). In *Limnology of Parakrama Samudra - Sri Lanka* : 165-170. Schiemer, F. (ed.) The Hague : Dr. W. Junk.

Drenner, R.W., Vinyard, G.L., Gophen, M. & McComas, S.R. (1982). Feeding behaviour of the cichlid, *Sarotherodon galilaeum* : selective predation on Lake Kinnert zooplankton. *Hydrobiologia*. 87 : 17-20.

Drenner, R.W., Mummert,J.R., de Noyelles, F. & Kettle, D. (1984a). Selective particle ingestion by a filter feeding fish and its impact on phytoplankton community structure. *Limnol. Oceanogr.* 29 (5) : 941-948.

Drenner, R.W., Taylor, S.B., Lazzaro, X. & Kettle, D. (1984b). Particle-grazing and plankton community impact of an omnivorous cichlid. *Trans. Am. Fish. Soc.* 113 : 397-402.

Drenner, R.W., Vinyard, G.L., Hambright, K.D. & Gophen, M. (1987). Particle ingestion by *Tilapia galilaea* is not affected by removal of gill rakers and microbranchiospines. *Trans. Am. Fish. Soc.* 116 : 272-276.

Drury, R.A.B. & Wallington, E.A. (1980). *Carleton's histological technique*. 5th edn. Oxford : Oxford University Press.

Durbin, A.G. & Durbin, E.G. (1975). Grazing rates of the Atlantic menhaden *Brevoortia tyrannus* as a function of particle size and concentration. *Mar. Biol., Berlin* 33 : 265-277.

Durbin, A.G. (1979). Food selection by plankton feeding fishes. In *Predator-prey systems in fisheries management* : 203-218. Stroud, R.H. & Cleeper, H. (Eds). Washington : Sport Fish. Inst..

Ebeling, A.W. (1957). The dentition of Eastern Pacific mullets, with special reference to adaptation and taxonomy. *Copeia* **1957** : 173-185.

Fink, W.L. (1981). Ontogeny and phylogeny of tooth attachment modes in actinopterygian fishes. *J. Morphol.* **167** : 167-184.

Fish, G.R. (1951). Digestion in *Tilapia esculenta*. *Nature, Lond.* **167** : 900-901.

Flood, P.R. (1978). Filter characteristics of appendicularian food catching nets. *Experientia.* **34** : 173-175.

Flood, P.R. & Fiala - Médioni, A. (1979). Filter characteristics of ascidian food trapping mucous films. *Acta Zool., Stockh.* **60** : 271-272.

Flood, P.R. & Fiala - Médioni, A. (1981). Ultrastructure and histochemistry of the food trapping mucous film in Benthic filter-feeders (Ascidians). *Acta Zool., Stockh.* **62** (1) : 53-65.

Flood, P.R. (1981). On the ultrastructure of mucus. *Biochem. Res.* **2** : 49-53.

Fogg, G.E., Stewart, W.D.P., Fay, P. & Walsby, A.E. (1973). *The Blue-green Algae*. London : Academic Press.

Friedland, K.D., Haas, L.W. & Merriner, J.V. (1984). Filtering rates of juvenile menhaden, *Brevoortia tyrannus* (Pisces : Clupeidae), with consideration of the effects of detritus and swimming speed. *Mar. Biol.* **84** : 109-117.

Friedland, K.D. (1985). Functional morphology of the branchial basket structures associated with feeding in the Atlantic menhaden, *Brevoortia tyrannus* (Pisces : Clupeidae), *Copeia* **1985** (4) : 1018-1027.

Frost, B.W. (1972). Effects of size and concentration of food particles on the feeding behaviour of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.* **17** (6) : 805-815.

Frost, B.W. (1975). A threshold feeding behaviour in *Calanus pacificus*. *Limnol. Oceanogr.* **20** : 263-266.

Fryer, G. & Iles, T.D. (1972). *The cichlid fishes of the great lakes of Africa : Their biology and evolution.*. Edinburgh : Oliver & Boyd.

Ganf, G.G. & Viner, A.B. (1973). Ecological stability in a shallow equatorial lake (L. George, Uganda). *Proc. R. Soc. Lond.* **184** : 321-346.

Gannon, J.E. (1976). The effects of differential digestion rates on zooplankton by Alewife, *Alosa pseudoharengus*, on determinations of selective feeding. *Trans. Am. Fish. Soc.* **105** : 89-95.

Geller, W. (1975). Die Nahrungsaufnahme von *Daphnia pulex* in Abhängigkeit von der Futterkonzentration, der Temperatur, der Körpergrösse und dem Hungerzustand der Tiere. *Arch Hydrobiol. Suppl.* **48** : 47-107.

Gerristen, J. & Porter, K.G. (1982). The role of surface chemistry in filter feeding by zooplankton. *Science.* **216** : 1225-1227.

Getachew, T. (1987). A study of an herbivorous fish, *Oreochromis niloticus* L., diet and its quality in two Ethiopian Rift Valley lakes, Awasa and Zwai. *J. Fish. Biol.* **30** : 439-449.

Gibson, R.N. (1988). Development, morphometry and particle retention capability of the gill rakers in the herring, *Clupea harengus* L. *J. Fish. Biol.* **32** : 949-962

Gophen, M. (1980). Food sources, feeding behaviour and growth rates of *Sarotherodon galilaeus* (Linnaeus) fingerlings. *Aquaculture.* **20** : 101-115.

Gosse, J.P. (1956). Dispositions speciales de l'appareil branchial des *Tilapia* et *Citharinus. Annls Soc. r. zool. Belg.* **86** : 303-308.

Greenwood, P.H. (1953). Feeding mechanisms of the cichlid fish *Tilapia esculenta* Graham. *Nature, Lond.* **172** : 207-208.

Greenwood, P.H. (1976). A review of the Family Centropomidae (Pisces, Periformes). *Bull. Br. Mus. Nat. Hist.* (Zool.). **29** : 1-81.

Hayes, B. & Fitzke, F. (1987). Design and use of a semi-automatic image analysis system. *Lab. Prac.* **36** (5) : 78-79.

Hofer, R. & Schiemer, F. (1983). Feeding ecology, assililation efficiencies and energetics of two herbivorous fish : *Sarotherodon* (Tilapia) *mossambicus* (Peters) and *Puntius filamentasus* (Cuv. et val.). In *Limnology of Parakrama Samudra - Sri Lanka* : 155-164. Schiemer, F. (ed.). The Hague : Dr. W. Junk.

Holling, C.S. (1959). The components of predation as revealed by a study of small mammal predation of the European Pine sawfly. *Canad. Entomol.* **91** : 293-332.

Holling, C.S. (1965). The functional response of predators to prey density and its role in mimicry and population regulation. *Mem. Entol. Soc. Can.* **45** : 5-60.

Holling, C.S. (1966). The functional response of invertebrate predators to prey density. *Mem. Entomol. Soc. Can.* **48** : 1-86.

Hossler, F.E., Ruby, J.R. & McIlwain, T.D. (1979). The gill arch of the mullet, *Mugil cephalus,* I surface ultrastructure. *J. Exp. Zool.* **208** : 379-398.

Hughes, E.D., Gorham, P.R. & Zehnder, A. (1958). Toxicity of a unialgal culture of *Microcystis aeruginosa. Can. J. Microbiol.* **4** : 225-236.

Hughes, G.M. & Wright, D.E. (1970). A comparative study of the ultrastructure of the water-blood pathway in the secondary lamellae of teleost and elasmobranch fishes. Benthic forms. *Z. Zellforsch.* **104** : 478-493.

Hughes, G.M. (1979). Scanning electron microscopy of the respiratory surfaces of trout gills. *J. Zool., Lond.* **187** : 443-453.

Humbert, W., Kirsch, R. & Meister, M.F. (1984). Scanning electron microscopic study of the oesophageal mucous layer in the eel, *Anguilla anguilla* L.. *J. Fish Biol.* **25** : 117-122.

Huysseune, A. (1983). Observations on tooth development and implantation in the upper pharyngeal jaws in *Astatotilapia elegans* (Teleostei, Cichlidae). *J. Morph.* **175** : 217-234.

Hyatt, K.D. (1979). Feeding strategy. In *Fish physiology VIII. Bioenergetics and growth*, 71-113. Hoar, W.S. Randall, D.J. & Brett, J.R. (Eds). London : Academic Press.

Hynes, H.B.N. (1950). The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes. *J. Anim. Ecol.* 19 : 36-58.

Hyslop, E.J. (1980). Stomach contents analysis - a review of methods and their application. *J. Fish Biol.* 17 : 411-429.

Iravani, J. & Melville, G.N. (1974). Long-term effect of cigarette smoke on mucociliary function in animals. *Respiration* 31 : 358-366.

Ismail, M.H., Verraes, W. & Huysseune, A. (1982). Developmental aspects of the pharyngeal jaws in *Astatotilapia elegans* (Trewavas, 1933) (Teleostei : Cichlidae). *Neth. J. Zool.* 32 : 513-543.

Ives, K.J. (1959). The significance of surface electric charge on algae in water purification. *J. Biochem. Microbiol. Tech. Eng.* 1 (1) : 37-47.

Ivlev, V.S. (1961). *Experimental ecology of the feeding of fishes*. Yale University.

Iwai, T. (1964). A comparative study of the taste buds in gill rakers and gill arches of Teleostean fishes. *Bull. Misaki Mar. Biol. Inst.*. 7 : 19-34.

Iwata, K. (1976). Morpholgical and physiological studies on the phytoplankton feeders in cyprinid fishes. I. Developmental changes of feeding organs and ingestion rates in Kawachibuna (*Carassius aurarus cuvieri*), silver carp (*Hypophthalmichthys molitrix*) and nigorobuna (C. *auratus grandoculis*). *Jap. J. Limnol.* 37 (4) : 135-147.

Johnson, D.W. (1973). Endocrine control of hydromineral balance in teleosts. *Am. Zool.* 13 : 799-818.

Johnsen, P.B. & Adams, M.A. (1986). Chemical feeding stimulants for the herbivorous fish, *Tilapia zillii*. *Comp. Biochem. Physiol. (A)* 83 : 109-112.

Jones, R., Bulduc, P. & Reid, L. (1973). Goblet cell glycoprotein and tracheal gland hypertrophy in rat airways : the effect of tobacco smoke with or without the anti-inflammatory agent phenylmethyloxadiazole. *Br. J. exp. Path.* 54 : 229-239.

June, F.C. & Carlson, F.T. (1971). Food of young Atlantic menhaden, *Brevoortia tyrannus*, in relation to metamorphosis. *Fish. Bull. U.S.* 68 : 493-512.

Kendall, M.W. & Dale, J.E. (1979). Scanning and transmission electron microscopic observations of rainbow trout (*Salmo gairdneri*) gill. *J. Fish Res. Bd. Can.* 36 : 1072-1079.

Kenny, J.S. (1969a). Feeding mechanisms in anuran larvae. *J. Zool. Lond.* 157 : 25-246.

Kenny, J.S. (1969b). Pharyngeal mucus secreting epithelia of anuran larvae. *Acta zool., Stockh.* 50 : 143-153.

King, D.P.F. & MacLeod, P.R. (1976). Comparison of the food and the filtering mechanism of pilchard *Sardinops ocellata* and anchovy *Engraulis capensis* off south west Africa, 1971-1972. *Invest. Rep. Sea Fish. Brch Repub. S. Afr.* No. 111 : 1-29.

Koehl, M.A.R. & Strickler, J.R. (1981). Copepod feeding currents : Food capture at low Reynolds number. *Limnol. Oceanogr.* 26 (6) : 1062-1073.

LaBarbera, M. (1978). Particle capture by a Pacific brittle star : experimental test of the aerosol suspension feeding model. *Science, N.Y.* 201 : 1147-1149.

LaBarbera, M. (1984). Feeding currents and particle capture mechanisms in suspension feeding animals. *Amer. Zool.* 24 : 71-84.

Lagler, K.F., Bardach, J.E. & Miller, R.R. (1977). *Ichthyology*. London : Wiley & Sons.

Lam, R.K. & Frost, B.W. (1976). Model of copepod filtering response to changes in size and concentration of food. *Limnol. Oceanogr.* 21 (4) : 490-500.

Langler, K.F. (1956). *Freshwater Fishery Biology*. Dubuque, Iowa : Wm. C. Brown.

Lanzig, W.J.R. & Higginbotham, D.R. (1976). Scanning microscopy of pharyngeal and oral teeth of the teleost *Tilapia mossambica* (Peters). *Hydrobiologia* 48 (2) : 137-139.

Law, J.H. (1960). Glycolipids. *Ann. Rev. Biochem.* 29 : 131-150.

Lawrence, S.G., Malley, D.F., Findlay, W.J., MacIver, M.A. & Delbaere, I.L. (1987). Method for estimating dry weight of freshwater planktonic crustaceans from measures of length and shape. *Can. J. Fish Aquat. Sci.* 44 (1) : 264-274.

Lazzaro, X. (1987). A review of planktivorous fishes : Their evolution, feeding behaviours, selectivities and impacts. *Hydrobiologia.* 146 : 97-167.

Leatherland, J.F. & Lam, T.J. (1969). Effect of prolactin on the density of mucous cells on the gill filaments of the marine form (trachurus) of the threespine stickleback, *Gasterosteus aculeatus* L. *Can. J. Zool.* 47 : 787-792.

Lehman, J.T. (1976). The filter-feeder as an optimal forager, and the predicted shapes of feeding curves. *Limnol. Oceanogr.* 21 (4) : 501-516.

Lewis, R.W. (1970). Fish cutaneous mucus : a new source of skin surface lipid. *Lipids* 5 : 947-949.

Liem, K.F. & Osse, J.W.M. (1975). Biological versatility, evolution, and food resource exploitation in African cichlid fishes. *Am. Zool.* 15 : 427-454.

Loder, T.C. & Liss, P.S. (1985). Control by organic coatings of the surface charge of estuarine suspended particles. *Limnol. Oceanogr.* 30 (2) : 418-421.

Lowe - McConnell, R.H. (1975). *Fish communities in tropical freshwaters.* New York : Longman.

Maitipe, P. & De Silva, S.S. (1985). Switches between zoophagy, phytophagy and detritivory of *Sarotherodon mossambicus* (Peters) populations in twelve man-made Sri Lankan lakes. *J. Fish Biol.* 26 : 49-61.

Mallatt, J. (1979). Surface morphology and functions of pharyngeal structures in the larval lamprey *Petromyzon marinus*. *J. Morp.* 162 : 249-274.

Mallatt, J. (1981). The suspension feeding mechanism of the larval lamprey *Petromyzon marinus*. *J. Zool. Lond.* 194 : 103-142.

Manion, P.J. (1967). Diatoms as food of larval sea lampreys in a small tributary of northern Lake Michigan. *Trans. Am. Fish. Soc.* 96 : 224-226.

Marin, V., Huntley, M.E. & Frost, B. (1986). Measuring feeding rates of pelagic herbivores : Analysis of experimental design and methods. *Marine Biology* 93 : 49-58.

Marshall, W.S. (1976). Effects of hypophysectomy and ovine prolactin on the epithelial mucus-secreting cells of the Pacific staghorn sculpin, *Leptocottus armatus* (Teleostei : Cottidae). *Can. J. Zool.* 54 : 1604-1609.

Mattey, D.L., Morgan, M. & Wright, E.E. (1980). A scanning electron microscope study of the pseudobranchs of two marine teleosts. *J. Fish Biol.* 16 : 331-343.

Mattheij, J.A.M. & Sprangers, J.A.P. (1969). The site of prolactin secretion in the adenohypophysis of the stenohaline teleost *Anoptichthys jordani*, and the effects of this hormone on mucous cells. *Z. Zellforsch.* 99 : 411-419.

McAndrew, B.J. & Majumdar, K.C. (1983). Tilapia stock identification using electrophoretic markers. *Aquaculture* 30 : 249-261.

McClatchie, S. & Lewis, M.R. (1986). Limitations of grazing rate equations : The case for time-series measurements. *Mar. Biol.* 92 : 135-140.

McDonald, M.E. (1985). Carbon budgets for a phytoplanktivorous fish fed three different unialgal populations. *Oecologia (Berl.)*. 66 : 246-249.

McMahon, J.W. & Rigler, F.H. (1963). Mechanisms regulating the feeding rate of *Daphnia magna* Straus. *Can. J. Zool.* 41 : 321-332.

Monod, T. (1961). *Brevoortia* Gill 1861 et *Ethmalosa* Regan 1917. *Bull. de l'Inst. Francais d'Afrique Noire.* 23 (A) : 506-547.

de Moor, F.C., Wilkinson, R.C. & Herbst, H.M. (1986). Food and feeding habits of *Oreochromis mossambicus* (Peters) in hypertrophic Hartbeespoort Dam, South Africa. *S. Afr. J. Zool.* 21 : 170-176.

Moore, J.W. & Beamish, F.W.H. (1973). Food of larval sea lamprey (*Petromyzon marinus*) and American brook lamprey (*Lampetra lamottai*). *J. Fish. Res. Bd. Can.* 30 : 7-15.

Moriarty, D.J.W. (1973). The physiology of digestion of blue-green algae in the cichlid fish *Tilapia nilotica*. J. Zool., Lond. 171 : 25-39.

Moriarty, C.M. & Moriarty, D.J.W. (1973). Quantitative estimation of the daily ingestion of phytoplankton by *Tilapia nilotica* and *Haplochromis nigripinnis* in Lake George, Uganda. *J. Zool. Lond.* 171 : 15-23.

Moriarty, D.J.W., Darlington, J.P.E.C., Dunn, I.G., Moriarty, C.M. & Tevlin, M.P. (1973). Feeding and grazing in Lake George, Uganda. *Proc. R. Soc. Lond.* B. 184 : 299-319.

Motta, P.J. (1986). A quantitative analysis of ferric iron in butterflyfish teeth (Chaetodontidae, Perciformes) and the relationship to feeding ecology. *Can. J. Zool.* 65 : 106-112.

Mullin, M.M., Stewart, E.F. & Fuglister, F.J. (1975). Ingestion by planktonic grazers as a function of concentration of food. *Limnol Oceanogr.* 20 (2) : 259-262.

Mummert, J.R. & Drenner, R.W. (1986). Effect of fish size on the filtering efficiency and selective particle ingestion of a filter-feeding clupeid. *Trans. Am. Fish. Soc.* 115 : 522-528.

Neihof, R.A. & Loeb, G.I. (1972). The surface charge of particulate matter in seawater. *Limnol Oceanogr.* 17 : 7-16.

Nilsson, S. (1984). Innervation and pharmacology of the gills. In *Fish Physiology* Vol. X : 185-227. Hoar, W.S. & Randall, D.J. (eds.). New York : Academic Press.

Nival, P. & Nival, S. (1976). Particle retention efficiencies of a herbivorous copepod, *Acartia clausi* (adult and copepodite stages) : effects on grazing. *Limnol. Oceanogr.* 21 (1) : 24-38.

Northcott, M.E. & Beveridge, M.C.M. (1988). The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (*Oreochromis niloticus*). *J. Zool., Lond.* 215 : 133-149.

O'Connell, C.P. (1981). Development of organ systems in the northern anchovy, *Engraulis mordax*, and other teleosts. *Am. Zool.* 21 : 429-446.

Olivereau, M. & Lemoine, A.M. (1972). Effets des variations de la salinité externe sur la teneur en acide N-acétyl-neuraminique (ANAN) de la peau chez l'anguille. *J. Comp. Physiol.* 79 : 411-422.

Olson, K.R. & Fromm, P.O. (1973). A scanning electron microscope study of secondary lamellae and chloride cells of rainbow trout (*Salmo gairdneri*). *Z. Zellforsch.* 143 : 439-449.

Orvig, T. (1967). Phylogeny of tooth tissues : Evolution of some calcified tissues in early vertebrates. In *Structural and chemical organisation of teeth* : 45-100. Miles, A.E.W. (ed.). London : Academic Press.

Paffenhöfer, G.A., Strickler, J.R. & Alcaraz, M. (1982). Suspension-feeding by herbivorous calanoid copepods : a cinematographic study. *Mar. Biol.* 67 : 193-199.

Pantastico, J.B., Espegadera, C. & Reyes, D. (1982). Fry -to - fingerling production of *Tilapia nilotica* in aquaria using phytoplankton as natural feed. *Philipp. J. Biol.* 11 (2-3) : 245-254.

Peters, R.H. (1984). Methods for the study of feeding, grazing and assimilation by zooplankton. In *A Manual on Methods for the Assessment of Secondary Productivity in Freshwaters* 2nd edn. (IBP Handbook 17) : 336-412. Downing, J.A. & Rigler, F.H. (eds.). Oxford : Blackwekk Scientific Publications.

Peters, R.H. & Downing, J.A. (1984). Empirical analysis of zooplankton filtering and feeding rates. *Limnol. Oceanogr.* 29 (4) : 763-784.

Philippart, J-Cl. & Ruwet, J-Cl. (1982). Ecology and distribution of tilapias. In *The Biology and Culture of Tilapias*. ICLARM Conference Proceedings 7 : 15-59. Pullin, R.S.V. & Lowe-McConnell, R.H. (eds.). International Center for Living Aquatic Resources Management, Manila, Philippines.

Pickering, A.D. (1974). The distribution of mucous cells in the epidermis of the brown trout *Salmo trutta* (L.) and the char *Salvelinus alpinus* (L.). *J. Fish Biol.* 6 : 111-118.

Pierce, B.A. (1983). Biotic effects of grazing by Tilapia (*Oreochromis aureus*) on phytoplankton populations in solar silos. *J. Freshwater Ecol.* 2 (3) : 293-304.

Pillay, T.V.R. (1952). A critique of the methods of study of food of fishes. *J. Zool. Soc. India.* 4 : 185-200.

Porter, K.G. (1977). The plant-animal interface in freshwater ecosystems. *Am. Sci.* 65 : 159-170.

Porter, K.G., Feig, Y.S. & Vetter, E.F. (1983). Morphology, flow regimes, and filtering rates of *Daphnia*, *Ceriodaphnia*, and *Bosmina* fed natural bacteria. *Oecologia* 58 : 156-163.

Prescott, G.W. (1969). *The algae : a Review*. London : Nelson.

Reeve, M.R. & Walter, M.A. (1977). Observations on the existence of lower threshold and upper critical food concentrations for the copepod *Acartia tonsa* Dana. *J. Exp. Mar. Biol. Ecol.* 29 : 211-221.

Reutter, K., Breipohl, W, & Bijvank, G.J. (1974). Taste bud type in fishes. 2. Scanning electron microscopical investigations on *Xiphophorus helleri* Heckel (Poeciliidae, Cyprinodontiformes, Teleostei). *Cell. Tissue Res.* 153 : 151-165.

Reutter, K. (1980). SEM-study of the mucus layer on the receptor field of fish taste buds. In *Olfaction and taste VII*. Van der Stare, H. (Ed). London : IRL Press Ltd.

Reynolds, C.S. (1984). *The Ecology of Freshwater Phytoplankton*. Cambridge : Cambridge University Press.

Richards, S. (1963). The demersal fish populations of Long Island Sound. *Bull. Bingham oceanogr. Collect. Yale Univ.* **18** : 5-101.

Rigler, F.H. (1961). The relation between concentration of food and feeding rate of *Daphnia magna* Straus. *Can. J. Zool.* **39** : 857-868.

Robertson, S.B. & Frost, B.W. (1977). Feeding by an omnivorous planktonic copepod *Aetideus divergens* Bradford. *J. exp. mar. Biol. Ecol.* **29** : 231-244.

Robotham, P.W.J. (1982). An analysis of a specialized feeding mechanism of the spined loach, *Cobitis taenia* (L.), and a description of the related structures. *J. Fish Biol.* **20** : 173-181.

Rosen, R.A. & Hales, D.C. (1981). Feeding of Paddlefish, *Polyodon spathula*. *Copeia* **1981** (2) : 441-455.

Ross, L.G. & Geddes, J.A. (1979). Sedation of warm water fish species in aquaculture research. *Aquaculture* **16** : 183-186.

Rubenstein, D.I. & Koehl, M.A.R. (1977). The mechanisms of filter feeding : some theoretical considerations. *Am. Nat.* **111** : 981-994.

Savage, R.M. (1952). Ecological,physiological and anatomical observations on some species of anuran tadpoles. *Proc. zool. Soc. Lond.* **122** : 467-514.

Saxena, M. & Kulshrestha, S.K. (1981). Histochemical studies of mucosubstances in the epidermis of a non scaly teleost *Mystus (mystus) vittatus* B1. (Pisces-Bagridae). *Histochemistry* **72** : 155-160.

Schmidt, W.J. (1969). Die Schmelznatur des eisnoxydhaltigen Kappe auf Teleostier-Zähnen. *Z. Zellforsh. mikrosk. Anat.* 93 : 447-450.

Schroll, F. (1959). Zur Ernahrungsbiologie der Steirischen Ammocoten *Lampetra planeri* (Bloch) und *Endontomyzon danfordi* (Regan). *Int. Rev. Ges. Hydrobiol.* 44 : 395-429.

Seale, D.B. & Wassersug, R.J. (1979). Suspension feeding dynamics of anuran larvae related to their functional morphology. *Oecologia (Berl.)* 39 : 259-272.

Seale, D.B. & Beckvar, N. (1980). The comparative ability of anuran larvae (genera : *Hyla, Bufo,* and *Rana*) to ingest suspended blue-green algae. *Copeia* 1980 : 495-503.

Seale, D.B., Hoff, K. & Wassersug, R. (1982). *Xenopus laevis* larvae (Amphibia, Anura) as model suspension feeders. *Hydrobiologia* 87 : 161-169.

Shaeffer, B. (1977). The dermal skeleton in fishes. In *Problems in vertebrate evolution - Linn. Soc. Symp. Series* 4 : 25-52. Andrews, S.M., Miles, R.S. & walker, A.D. (eds.).

Shen, S.C. (1969). Comparative study of the gill structure and feeding habits of the anchovy *Engraulis japonica* (Hout). *Bull. Inst. Zool. Acad. Sinica* 8 : 21-35.

Sibbing, F.A. & Uribe, R. (1985). Regional specializations in the oro-pharyngeal wall and food processing in the carp (*Cyprinus carpio* L.). *Neth. J. Zool.* 35 : 377-422.

Sinha, G.M. (1975). A histochemical study of the mucous cells in the bucco-pharyngeal region of four Indian freshwater fishes in relation to their origin, development, occurrence and probable functions. *Acta histochem. Bd.* 53 : 217-223.

Solanki, T.G. & Benjamin, M. (1982). Changes in the mucous cells of the gills, buccal cavity and epidermis of the nine-spined stickleback, *Pungitius pungitius* L. induced by transferring the fish to sea water. *J. Fish Biol.* 21 : 563-575.

Spataru, P. (1976). The feeding habits of *Tilapia Galilaea* (Artedi) in Lake Kinneret (Israel). *Aquaculture* 9 : 47-59.

Stanier, R.Y., Kunizawa, R., Mandel, M., Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae. *Bact. Rev.* 35 : 171-205.

Steele, J.H. (1974). *The structure of marine ecosystems*. Harvard University.

Stiassny, M.L.J. (1981). The phyletic status of the family Cichlidae (Pisces, Perciformes) : A comparative anatomical investigation. *Neth. J. Zool.* 31 : 275-314.

Stiassny, M.L.J. (1982). The relationships of the neotropical genus *Cichla* (Perciformes, Cichlidae) : A phyletic analysis including some functional considerations. *J. Zool., Lond.* 197 : 427-453.

Strickler, J.R. (1982). Calanoid copepods, feeding currents, and the role of gravity. *Science* 218 : 158-160.

Sutterlin, A.M. & Saunders, R.L. (1969). Proprioceptors in the gills of teleosts. *Can. J. Zool.* 47 : 1209-1212.

Suyehiro, Y. (1942). A study on the digestive system and feeding habits of fish. *Jpn. J. Zool.* 10 : 1-303.

Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia.*. *Publs Br. Mus. nat. Hist.* No. 878, 1-583.

Vinyard, G.L., Drenner, R.W., Gophen, M., Pollingher, U., Winkelman, D.L. & Hambright, K.D. (1988). An experimental study of the plankton community impacts of two omnivorous filter-feeding cichlids, *Tilapia galilaea* and *Tilapia aurea.* *Can. J. Fish. Aquat. Sci.* 45 (4) : 685-690.

Vollenweider, R.A. (1974). *A Manual on Methods For Measuring Primary Production in Aquatic Environments* (IBP Handbook 12). Oxford : Blackwell.

Wassersug, R. (1972). The mechanisms of ultraplanktonic entrapment in anuran larvae. *J. Morph.* 137 : 279-288.

Wassersug, R. & Hoff, K. (979). A comparative study of the buccal pumping mechanism of tadpoles. *Biol. J. Linn. Soc.* 12 : 225-259.

Weatherley, A.H. (1972). *Growth and Ecology of Fish Populations.* London : Academic Press.

White, P.N. & Bruton, M.N. (1983). Food and feeding mechanisms of *Gilchristella aestuarius* (Pisces : Clupeididae). *S. Afr. J. Zool.* 18 : 31-36.

Whitehead, P.J. (1959). Feeding Mechanism of *Tilapia nigra.* *Nature, Lond.* 184 : 1509-1510.

Whyte, S.A. (1975). Distribution, trophic relationships and breeding habits of the fish populations in a tropical lake basin (Lake Bosumtwi - Ghana). *J. Zool., Lond.* 177 : 25-56.

Williams, L.G. (1982). Mathematical analysis of the effects of particle retention efficiency on determination of filtration rate. *Mar. Biol.* **66** : 171-177.

Windell, J.T. (1968). Food analysis and rate of digestion. In *Methods for Assessment of Fish Production in Freshwaters* 1st edn. : 197-203. Ricker, W.E. (ed.). Oxford : Blackwell Scientific Publications.

Windell, J.T. & Bowen, S.H. (1978). Methods for study of fish diets based on analysis of stomach contents. In *Methods for Assessment of Fish Production in Freshwaters* 3rd edn. : 219-226. Bagnel, T. (ed.). Oxford : Blackwell Scientific Publications.

Wittouck, P.J. (1975). Action de la prolactine sur les cellules á mucus épidermiques chez l'Axolotl intact et hypophysectomisé. *Gen. Comp. Endocrinol.* **27** : 254-261.

Wright, D.J. O'Brien, W.J. & Luecke, C. (1983). A new estimate of zooplankton retention by gill rakers and its ecological significance. *Trans. Am. Fish. Soc.* **112** : 638-646.

Yamada, K. & Yokote, M. (1975). Morphometrical analysis of mucosubstances in some epithelial tissues of the eel (*Anguilla japonica*). *Histochemistry* **43** : 161-172.

Yashouv, A. & Chervinski, J. (1960). Evaluation of various food items in diet of *T. nilotica*. *Bamidgeh* **12** (3) : 71-78.

Yashouv, A. & Chervinski, J. (1961). The food of *Tilapia nilotica* in ponds of the Fish Culture Research Station at Dor. *Bamidgeh* **13** (2) : 33-39.