STUDIES OF FILTER FEEDING CARPS OF COMMERCIAL IMPORTANCE IN BANGLADESH WITH PARTICULAR EMPHASIS ON THE USE OF AUTOMATED COUNTING METHODS

A THESIS SUBMITTED TO THE UNIVERSITY OF STIRLING FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

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Abstract

The filter feeding capabilities of the Indian major carps *Labeo rohita* (Ham.) and *Catla catla* (Ham.) and the Chinese carp *Hypophthalmichthys molitrix* (Val.) were studied under laboratory conditions. An ultrastructural and histological study of the pharyngeal apparatus associated with filter feeding of laboratory reared fish between 30 and 90 mm standard length (SL) showed that a large number of mucus cells, both small goblet and large clavate cells, were present in the bucco-pharyngeal region of all species studied and on the wall of the suprabranchial organ of *H. molitrix*. The density of taste buds was observed to be much greater on the roof of the pharynx and comparatively low on the roof of the mouth in all species. Among the carps studied, *H. molitrix* had the greatest concentration of taste buds on both the roof and floor of the bucco-pharyngeal region.

An important part of the study was the development of a sound methodology for counting algal cells and colonies. An automated electronic particle counter, the Coulter Multisizer, was used to increase the speed of counting. A study was also carried out to determine the effects of the preservatives Lugol's iodine and buffered formalin at different concentrations on both long-term and short-term storage of *Pediastrum boryanum*, *Microcystis aeruginosa* and *Chlorella vulgaris*. Both Lugol's iodine and buffered formalin of different concentrations caused significant decreases in the numbers of cells and colonies during preservation. Lugol's iodine of different

concentrations caused cell shrinkage in *C. vulgaris* and *M. aeruginosa* after 24 h of preservation.

The abilities of carp fry and fingerlings to ingest a range of algal species were investigated. *H. molitrix* was found to be the most efficient among the carps studied in ingesting *P. boryanum* and *M. aeruginosa*, although *C. catla* was found not to be able to ingest *M. aeruginosa*. Ingestion rate was found to be positively correlated with algal density. It was also observed that algal size had a positive effect on ingestion rates. In a separate trial it was found that silver carp did not ingest a toxic strain of *M. aeruginosa*.

An attempt was also made to evaluate whether these filter feeding carp could detect and ingest unattached bacteria by exposing them to the bacterium *Chromobacterium violaceum* in suspension. Examination of the gut contents of the exposed fish showed that the numbers of *H. molitrix* and *L. rohita* ingesting viable bacteria increased with the concentration of the bacterial suspension and with exposure time. However *C. catla* ingested far fewer bacteria per unit time than the other two species.

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I would like to thank my supervisor Dr Malcolm C M Beveridge for his advice, encouragement and worthy criticism throughout the course of this research and during the writing up of this thesis. I am also grateful for his patience and help during my problems.

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Finally I would like to thank my wife Happy, who helped me throughout the course of my research and all other activities in Scotland by offering her endless love. Thanks are also due to my daughter, son, father and mother for their help and encouragement.

DEDICATED

TO

MY FAMILY

DECLARATION

I hereby declare that the research work presented in this thesis was conducted by me and that this work has not been submitted for any other degree. All works in this thesis referred to have been duly acknowledged.

Signed

S.M. Rahmatullah

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Abbreviations used in plates

A anterior

b brain

D dorsal

e eye

f fold

gr gill raker

l lip

p papillae

po palatal organ

rv respiratory valve

so suprabranchial organ

t taste bud

Contents

CHAPTER I

Introduction

1.1 Evolution and extent of filter feeding among fishes

The oldest teleostean fishes evolved as generalized predators feeding on comparatively large prey (macrophagy) (Gosline, 1971). The main divergence from this basic pattern has been a trend towards feeding on smaller prey (microphagy), such as plankton. The evolution from macrophagy to microphagy is marked by the development of specialized feeding structures and the regression of others (Lazzaro, 1987). This has included, for example, modification of the jaw from fixed to protrusible, replacement of teeth by elaborate gill rakers on the branchial arches, modification of some gill rakers into an epibranchial organ on the roof of the mouth, and lengthening of the digestive tract in order to process larger amounts during the digestive pause between meals (Marshall, 1965; Nelson, 1967; Durbin and Durbin, 1975). The evolution, feeding behaviour and selectivityes of planktivorous fishes has been reviewed by Lazzaro (1987).

The most specialized of the microphagous feeders are the filter-feeding species. The menhaden, a filter-feeding clupeid microphage, repeats most of the evolutionary steps mentioned above during its life cycle, as do a number of other species. The selective predation of larval menhaden is directed towards individual zooplankton, mainly

copepods (June & Carlson, 1971), and during metamorphosis, particulate feeding is gradually replaced by filter-feeding on zooplankton, phytoplankton, and fine detritus (Durbin & Durbin, 1975).

Only a small proportion of the energy produced by phytoplankton can be transferred through the food web to the higher trophic levels, usually predatory macrophages. Hence a considerable proportion is available to plankton feeding fishes. Planktivores are involved in the main commercial fisheries of the world (anchovies, sardines, herrings, menhadens and many others). Today, teleosts include more than 95% of the living species of fish (Marshall, 1965) and microphagy is apparent in most (freshwater, at least) orders even in the Acipensiformes (Actinopterygia) (see Table 1.1).

Most fishes feed on plankton during at least some period of their lives. Some fishes feed on plankton during their entire lives, whereas others feed on plankton only during the early stages. Some fishes are obligate planktivores, feeding exclusively on plankton; others are facultative planktivores feeding on plankton as well as on other food items.

1.2 Cyprinids and filter-feeding

Cyprinids are generally known under the common names of carps, barbs, minnows, roach, rudd, dace, bitterling, rasbora, danios and gudgeon. Their dominant

Table 1.1 Examples of filter feeding fishes arranged according to Order, Family and Species.

Order	Family	Species	Reference
Acipensiformes	Polyodontidae	*Polyodon spathula*	Rosen and Hales,1981
Clupeiformes	Clupeidae	*Dorosoma cepedianum*	Drenner, 1977; Drenner *et al.*, 1978, Drenner and McComas, 1980.
		D. petenense	Holanov and Tash, 1978.
		Alosa pseudoharengus	Brooks and Dodson, 1965; Rasmussen, 1973; Janssen, 1976, 1980.
		A. astivalis	Brooks and Dodson, 1965; Janssen, 1982.
		Sardinops sagax	Nelson, 1979.
		Clupea harengus	Rosenthal and Hempel, 1970.
		Brevoortia tyrannus	June and Carlson, 1971; Durbin and Durbin, 1975.
		Sardinops ocellata	King and Mcleod, 1976.
	Engraulidae	*Engraulis mordax*	Leong and O'Connell, 1969; O'Connell, 1972; Nelson, 1979.
Cypriniformes	Cyprinidae	*Carassius auratus*	Hester, 1968.
		Cyprinus carpio	Grygierek *et al.*, 1966.
		H. molitrix	Lin, 1969; Kajak *et al.*, 1975.

Order	Family	Species	Reference
		L. rohita	Khan and Siddiqui, 1973; Khan and Jhingran, 1975; Jhingran and Pullin, 1985.
		C. catla	Alikunhi, 1952; Jafri and Mustufa, 1977.
		Aristichthys nobilis	Lin, 1969; Spataru *et al.*, 1983.
		Abramis brama	Lammens, 1985; Hoogenboezem *et al.*, 1990; Hoogenboezem *et al.*, 1991.
Gasterosteiformes	Gasterosteidae	*Gasterosteus aculeatus*	Gibson, 1980.
Perciformes	Centrarchidae	*Lepomis macrochirus*	Werner, 1972.
	Cichilidae	*Tilapia aurea*	Spataru and Zorn, 1978; Gophen *et al.*, 1983; Drenner *et al.*, 1984.
		T. esculenta	Greenwood, 1953.
		T. nilotica	Moriarty, 1973; Moriarty and Moriarty, 1973.
		Oriochromis niloticus	Northcott, 1988; Beveridge *et al.*, 1988; Northcott *et al.*, 1991.

characteristics are the lack of jaw teeth and the possession of a (usually) protrusile upper jaw and pharyngeal teeth. In all, there are some 1700 valid species belonging to at least 220 genera, making the family the most speciose of all freshwater teleost fishes. The cyprinids have a wide geographical distribution and occur in much of mainland Eurasia, Japan, most of the East Indian islands, Africa and North America (Howes, 1991). Cyprinids contributed some 32% of global aquaculture production (tonnes) in 1988 of which silver carp alone contributed 1.1% (FAO 1990). Among the cyprinids, there are a number of groups including the Indian major carp species such as *Labeo rohita* (Ham.), *Catla catla* (Ham.) and Chinese carp species (*e.g. Hypophthalmichthys molitrix* (Val.), *Aristichthys nobilis* (Richardson) which are of great economic importance in the fisheries and aquaculture industries of Bangladesh and other Asian countries.

Composite fish culture, or polyculture, of Indian major carps together with Chinese carps and the common carp has been found to result in high fish production in freshwater ponds (Sukumaran *et al.* 1968; Alikunhi *et al.*, 1971; Chaudhuri *et al.* 1975). At higher densities, however, there is growing evidence that the Chinese carps adversely affect the growth of the Indian major carps such as catla and rohu (Lakshmanan *et al.*,1971; Dey *et al.*1979). Although no specific studies have been undertaken, the dominance of Chinese carp over the Indian major carp is widely believed to be due to their greater competitive advantage in exploiting food resources.

Competition is reputedly greatest between the planktivorous species of carps such as between catla (*C. catla*) and bighead carp (*A. nobilis*) and between rohu (*L. rohita*) and silver carp (*H. molitrix*) (*e.g.* Dewan *et al.*, 1991).

1.3 Filter-feeding behaviour

Plankton feeding fishes shows two distinct behaviours with regard to feeding on plankton: particulate feeding and filter feeding. Particulate plankton feeding fishes attack single individual prey items which they visually select prior to engulfment from the water column (Werner, 1977; Janssen, 1980, 1981). By contrast, both pump filter feeders and tow-net filter feeders do not visually select individual prey items, but engulf a volume of water containing the food organisms, then remove the planktonic prey and particles by passing the water through filtering structures (*e.g.* gill rakers, microbranchiospines on the gill arches and branchial tooth plates). Tow-net filter feeders surround the prey items with their mouths which are held fully agape while swimming rapidly (Durbin and Durbin, 1975; Colin, 1976; Rosen and Hales, 1981). Pump filter feeders use rhythmic suction actions to capture prey items, while swimming slowly or remaining relatively stationary (Moriarty *et al.*, 1973; Drenner, 1977; Janssen, 1976).

Janssen (1976) described an intermediate feeding mode, gulping, for the alewife, *Alosa*

pseudoharengus, and the cisco, *Coregonus artedii*, where fish use short sequences of several pump actions which alternate with pauses of about 0.5 seconds.

1.4 Filter-feeding mechanisms

The use of intricate gill rakers in the filter feeding of planktivorous fishes has been well documented (Iwata, 1976; Shen, 1969; Hossler *et al.*, 1979; Cremer and Smitherman, 1980; O'Connell, 1981; Lammens, 1985; Lammens *et al.*, 1987; Hoogenboezem *et al.*, 1990, 1991; Northcott *et al.*, 1991). However, not all plankton feeding fishes possess well-developed gill-rakers. Suyehiro (1942) noted that a number of families contain planktivorous species which possess very coarse gill-rakers. The families Syngnathidae and Fistulariidae have totally degenerate gill-rakers, yet their chief foods are plankton and benthic diatoms. Many of the tilapias (Cichlidae) feed on plankton without the benefit of well developed gill-rakers (Fryer and Iles, 1972; Pauly, 1976; Northcott 1988; Northcott and Beveridge 1988.)

Some worker have believed that gill rakers functioned as passive sieves in filter feeding (Durbin and Durbin, 1975; Rosen and Hales, 1981; Drenner *et al.*, 1984; Mummert and Drenner, 1986; Hoogenboezem, 1991). Gill raker morphology of rohu, catla and silver carp suggests that the structures represent efficient passive sieves in view of their elaborate structure. Smith (1989) has also suggested that the gill rakers of silver carp act as passive, mechanical sieves during filter feeding.

Sanderson *et al.*, (1991) have proposed that the gill arch structure of another cyprinid, the blackfish, *Orthodon microlepidotus*, acts as a barrier during feeding which directs particle-laden water towards the mucus covered roof of the oral cavity, where particles are retained. Gill-arch structures had previously been assumed to be the site of particle retention in suspension feeding fishes. Flow velocity of water and its direction inside the oral cavity of vertebrate suspension feeders, particularly at the level of filtering elements, is important. Labarbera (1984) suggested that virtually all suspension feeders capture particles from the water at low Reynolds number with cylindrical filtering elements. Gerristen and Porter (1982) observed that the surface chemistry of filtering appendages as well as food particles are important for the crustacean zooplankter *Daphnia magna* feeding at low Reynolds numbers.

Other than sieving, the flow of water and its direction within the oral cavity, and cylindrical filtering elements, Greenwood (1953) has proposed mucus entrapment of phytoplankton in the filter feeding mechanism of *Oreochromis esculentus*. Mucus entrapment mechanisms in suspension feeding have subsequently been suggested for other fishes (Robotham, 1982; Sibbing and Uribe, 1985; Beveridge *et al.*, 1988; Northcott and Beveridge, 1988; Sanderson *et al.*, 1991). The presence of large numbers of mucus cells in the bucco-pharyngeal region of rohu and catla has been reported (Kapoor, 1957; Sinha, 1975; Sinha and Moitra, 1975).

Filter feeding fish do not visually detect individual prey items, but engulf a volume of water containing the food organisms along with non-food items. Taste buds on the oro-pharyngeal surface help the fish to select food from non-food particles during filter feeding. The role of taste buds in selection and detection of food particles in fishes has been proposed by several workers (Sibbing and Uribe, 1985; Sibbing, 1987; Hlohowskyj *et al.*, 1989).

The results of quantitative filter feeding studies have been used to create functional response models. A number of curves have been fitted to data from filter feeding studies (Ivlev, 1961; Mullin *et al.*, 1975; Seale and Wassersug, 1979; Seale *et al.*, 1982; Kiørboe *et al.*, 1985; Drenner *et al.*, 1987). Kiørboe *et al.*, (1985) described a sigmoid curve which best fitted data on ingestion rates of a planktonic copepod. However, Mullin *et al.*, (1975) demonstrated statistically that all three curves (rectilinear, Ivlev and Michaelis-Menten) could be used justifiably to describe zooplankton data. Most of the work on the functional responses of filter feeding has been done on zooplankton, whereas little information exists on filter feeding fish species.

Particles of different sizes and types, including free-living unattached bacteria, attached bacteria, phytoplankton (sometimes including toxic strains), zooplankton and suspended organic matter, occur in aquatic ecosystems (Bowen, 1976; Schroeder, 1978; Opuzynski, 1981a, Reynolds,1984). Adamek and Spittler (1984) reported that silver

carps are capable of ingesting particles of < 1 - 3.37 μm. Kuznetsov (1977) suggested that silver carp may be able to ingest bacteria from the water in association with algae. Detection and ingestion of unattached bacteria in suspension by a number of other fishes has also been reported (Beveridge *et al.*, 1989, 1991).

1.5 The present study

Given the above information and the lack of studies on filter-feeding carps it was decided to investigate the filter feeding mechanisms of the economically important species rohu, catla and silver carp in a series of experiments:

i) an histological and ultrastructural investigation of the bucco-pharyngeal region and the organs associated with filter feeding of rohu, catla and silver carp should be carried out in order to help describe and understand the filter feeding mechanisms involved.

ii) a quantitative study of filter feeding on phytoplankton of two sizes and five densities (9 - 193 μm^3) $x10^6$ bio-volume per ml should be undertaken in order to generate functional response curves. The chosen density were the more or less similar natural planktonic density at different season of the year of different freshwater habitat of Bangladesh. In addition, both toxic and non-toxic strains of *Microcystis aeruginosa* were used to assess the filter feeding strategies of one

species (silver carp) in the presence of toxic algae.

iii) a study on the ingestion of bacteria would be carried out to determine whether fishes were able to detect or ingest the unattached bacteria in suspension.

With regards to experiment (ii) in which large volumes of data are likely to be generated it was first necessary to develop and evaluate automated counting procedures for planktonic algae.

CHAPTER II

Investigation of automated particle counting and sizing methods for algae

2.1 Introduction

For various reasons, aquatic ecologists and others must often resort to working with preserved phytoplankton material and use automated counting and sizing methods in order to rapidly process large numbers of samples. In grazing experiments it is very important to obtain accurate counts, sizes and bio-volumes of algal cells in order to be able to interpret results.

There are several types of apparatus used for counting of algae, including the Lund chamber (Lund, 1959), haemacytometer and Sedgewick-rafter cell (Nalewajko, 1966; Bailey-Watts, 1978; Northcott *et al.*, 1991). In recent years electronic particle counters have been used in biological research for precise particle counting (Parsons, 1973; Vanderploeg, 1981; Breteler, 1985; Kersting, 1985; Robinson *et al.*, 1990).

There are several types of electronic particle counter in use, including the Coulter Multisizer (Coulter Electronics, Luton, Beds, U.K.). The Coulter Multisizer consists of three elements (Fig. 2.1): (a) the Multisizer unit, (b) sampling stand and (c) vacuum control unit. A cuvette containing the sample to be analyzed is placed in the platform of the sampling stand (Fig 2.3). A stirrer ensures that the sample is well-mixed. When directed, a quantity of the sample is drawn by vacuum pressure created by the pump and regulated by the vacuum control unit (Fig. 2.4) through a small, precisely drilled orifice into a glass tube. The tube is selected on the basis of particle size, each tube being able to deal with particles 2-60% of stated orifice size, although according to the manufacturer, particles 20-40% of orifice size give best results. As each particle passes through the tube aperture it creates an electric pulse by changing the resistance of the sensing zone, the size of the pulse being related to the size of the particle. The signal is transmitted to the Multisizer unit (Fig.2.2) where it is processed and displayed on the screen. The display (Fig. 2.5) gives information on N = particle count, $\underline{N}$ = particle count with coincidence correction (*i.e.* a statistical-based correction for two particles which may have passed through the tube orifice at the same time), Σ = channelizing count with particle size distribution. By manipulation of the displayed data it is possible to count only particles within a defined size range. The Multisizer can be operated in a variety of 'modes' in which particles in a known volume of sample are counted (siphon mode) or particle counting takes place over a defined period of time (time mode).

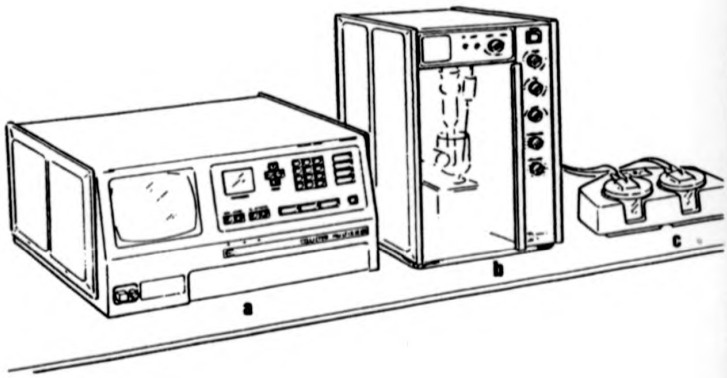


Fig. 2.1 The Coulter Multisizer (a) Multisizer; (b) sampling stand; (c) vacuum control unit (reproduced from Coulter manual)

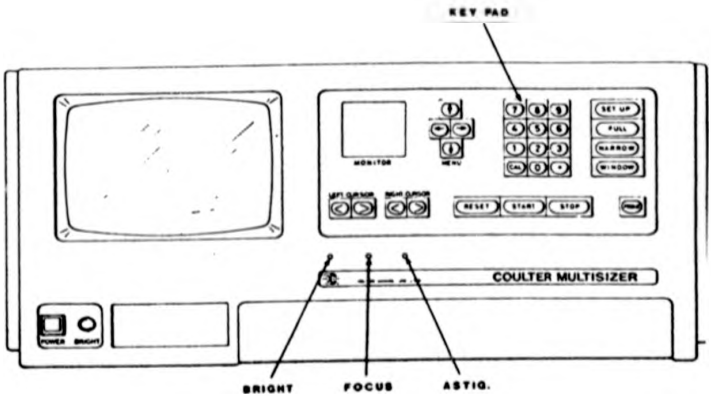


Fig. 2.2 Multisizer front view (Produced from Coulter manual)

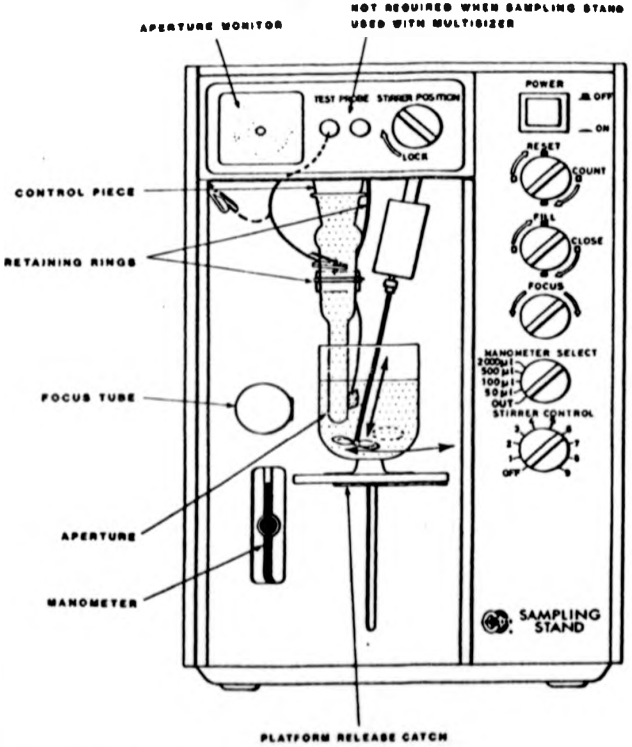


Fig. 2.3 Sampling stand front view (reproduced from Coulter manual)

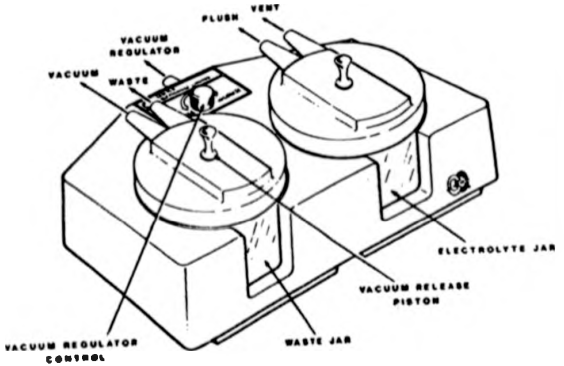


Fig. 2.4 Vacuum control unit (reproduced from Coulter manual)

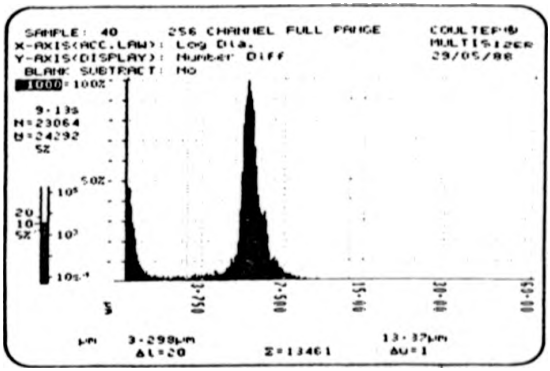


Fig. 2.5 Displayed data on the Multisizer screen (reproduced fror Coulter manual)

Detailed descriptions of electronic particle counters and the principles of operation are given in manufacturer's manuals (*e.g.* Coulter) and in more general texts (*e.g.* Parsons, 1973).

Whilst it is generally assumed that automated methods involve considerable savings in time, reports on the advantages and disadvantages of using electronic particle counters for algae are scant. Kersting (1985) reported that one problem of electronic particle counters (Coulter Electronics Ltd Model TA II, Model ZB and Channelizer C-1000 coupled to Model ZB) is count loss caused by dead time of the instruments (time elapsed between two electrical pulses at the maximum capacity of the machine to generate the electrical pulses per second). In order to avoid this, a maximum counting frequency of 200 counts/second was recommended.

Light and electron microscopy are widely used for determination of algal cell size (Bellinger, 1974; Sicko-Goad *et al.*, 1977; Clarke *et al.* 1987). Electronic particle counters are also used for this purpose (Sheldon and Parsons, 1967; Vanderploeg, 1981; Bakker *et al.*, 1985). The Multisizer expresses particle size in terms of equivalent spherical diameter (ESD), *i.e.* irrespective of particle size and shape the Multisizer expresses particle size in terms of an idealized sphere (Fig.2.6) and uses this to calculate bio-volume. Vanderploeg (1981) concluded that the bio-volume of long thin cells were significantly under-estimated by electronic particle counters unless cell

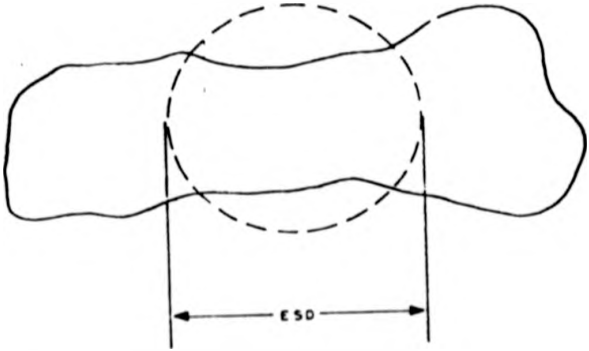


Fig 2.6 Equivalent Spherical Diameter (ESD) (reproduced from Coulter manual).

lengths were shorter than tube orifice size. Bakker *et al.* (1985) stated that using monocultures or single species-dominated natural phytoplankton, cell counts and bio-volume estimations obtained by visual and electronic methods show reasonable agreement. They also reported under-estimation of bio-volume by electronic counters when algae are cylindrical, elongate or needle-like.

Acid Lugol's iodine and buffered formalin are widely recommended as preservatives of freshwater algae (Vollenweider 1974; Wetzel and Likens 1979, APHA 1989), although little work has been done on their effects on algal cells and colonies. In a study of autotrophic and heterotrophic marine flagellates Breteler (1985) found drastic losses of cells and changes in cell size occurred when Lugol's iodine and other preservatives (*e.g.* acetic acid, trichloric acid, sublimate and benzoic acid-5hydroxy-sulfo) were used. Both Silver and Davoll (1978) and Holtby and Knoechel (1981) have reported the loss of radioisotope label as a result of algal cell disintegration in Lugol's iodine.

In this study it was decided to investigate the usefulness of automated counting and sizing methods for algal cells and to examine how numbers and sizes of cells and colonies of freshwater green algae (Chlorophycaea) and Cyanobacteria (blue-green algae), as determined by the Coulter Multisizer, are affected by both short-term and long-term storage in Lugol's iodine and buffered formalin. The investigation of automated counting and size and bio-volume estimation was carried outonly with *P.*

boryanum and *M. aeruginosa*, the two algae used in the ingestion experiments (see Chapter IV).

2.2 Materials and Methods

2.2.1 Algae

Cultures of the freshwater blue-green alga (Cyanobacteria) *Microcystis aeruginosa* Kutzing emend. Elenkin 1924 were obtained from the Department of Biology, University of Dundee. The green algae *Pediastrum boryanum* (Turpin) Meneghini 1840 and *Chlorella vulgaris* Beijerinck were obtained from the NERC Culture Collection of Algae and Protozoa, FBA, Windermere, U.K. *C. vulgaris*, *P. boryanum* and *M. aeruginosa* were cultured in Bold's Basal medium (Nichols 1973), Jaworski's medium and nitrate-supplemented BG-11 medium (Stanier *et al* 1971) respectively (see Appendix 1, 2, 3). Batch-culture methods were employed. Cultures were grown in 5 l flasks in an illuminated cabinet maintained at 16-19 °C.

2.2.1 Counting and estimation of cell size and bio-volume

In order to compare the performances of different counting techniques five replicate counts were made by haemacytometer BS 748 and Sedgewick-Rafter cell S50 (Pisons) for each of samples of *M. aeruginosa* and *P. boryanum* respectively. Standard Methods

(APHA 1989) were followed for counting the algae by haemacytometer and Sedgewick-rafter cell. The same samples were counted using the Coulter Multisizer, the methods employed being based on those described by Parsons (1973). Ten different count rates (count. s^{-1}) with five replicates were employed using siphon mode. Each replicate consisted of 200 µl sub-samples transferred to 20 ml recommended commercially-prepared electrolyte, a non-sterile but bacteriostatic, phosphate-buffered saline solution; concentration = 0.85% NaCl (ISOTON II; Coulter Electronics, Ltd., Luton, Beds., U.K.).

At least one hundred cells or colonies of each algal species were measured using an Olympus BH2 phase contrast light microscope. The bio-volumes of *M. aeruginosa* cells were calculated using the generalized formula for a sphere as Northcott *et al.*, (1991):-

$$V = 4/3\pi abc$$

where a = 0.5 x length; b = 0.5 x height; c = 0.5 x width. The bio-volume of *P. boryanum* was calculated by using the formula:-

$$V = \pi r^2 L$$

where r = radius of the colony; L = depth of the colony. The Coulter Multisizer was also used for estimating the size and bio-volume of the algae. A similar calculation for

P. boryanum was used by Bellinger (1974).

The size distribution of the algal cells was also determined by introducing the same volume (200 µl) of algae into 20 ml ISOTON II. Counts were determined immediately, and after 5 min, 10 min, 20 min and 40 min following transfer to ISOTON II.

2.2.3 Effects of preservatives on algal cells

Acid Lugol's iodine was prepared according to the methods given in Wetzel and Likens (1979) and Standard Methods (APHA 1989), in which 10 g iodine and 20 g potassium iodide are added to 200 ml distilled water containing 20 ml glacial acetic acid. Buffered formalin (20 g sodium borate + 1 l 37% formaldehyde) was prepared according to the method given in Standard Methods (APHA 1989). The solutions were stored in a refrigerator at 4°C and used within 3 weeks.

Four concentrations of each preservative and seven preservation periods of both short and long duration were investigated. Samples taken from log-phase cultures of algae were preserved with Lugol's iodine at concentrations of 0.05%, 0.1%, 0.2% and 0.4% and in buffered formalin at concentrations of 0.5%, 1%, 2% and 4 %. Three replicate samples were made for each concentration. Samples were stored at room temperature in ambient light conditions, away from direct sunlight.

Cell or colony numbers and their size distribution for each sample were estimated by Coulter Multisizer fitted with tubes of orifice size 70, and 200 μm. An initial count was made using fresh material. Distinct peaks of particle number versus particle size were generally observed, other parts of the spectrum being assumed to represent bacteria and cellular debris. Using the channel selection facility on the Multisizer it is possible to exclude unwanted counts and thus only the peak corresponding to algal cells or colonies was counted (see Figure 2.5). As background counts (*i.e.* counts of particles present in the electrolyte solution) were extremely low (70-90) in all cases, no adjustments to counts were made.

Four sub-samples from each sample were examined immediately prior to preservation and at intervals throughout the preservation period. For the purpose of analysis, 200 μl sub-samples from *C. vulgaris*, *M. aeruginosa* and *P. boryanum* were added to 20 ml of the recommended ISOTON II. All counts were made between 1 minute and 5 minutes after introduction to the electrolyte.

2.2.4 Statistical methods

Multifactor ANOVA was used to verify significant treatment effects and Duncan's multiple range test used to compare individual treatments within the ANOVA design. Student's t-test was used to verify differences between samples (Sokal and Rohlf, 1973; Bailey, 1981).

2.3 Results

Channelizing count loss (difference between total count and channelizing count) was estimated as 1% when the count was 200 - 300 particle, s^{-1} and 22% at 2600 - 2800 particle, s^{-1} count (Table 2.1). All subsequent sample counts were maintained at 200-300 particles, s^{-1} in order to minimise count losses.

Counts obtained from the Coulter Multisizer were slightly higher than those from the haemacytometer and Sedgewick-Rafter cell. A comparison of counts using different devices is presented in Table 2.2.

The diameter of *P. boryanum* measured by light microscopical method was higher than cell ESD measured by Coulter Multisizer. Bio-volume of *P. boryanum* measured by light microscopy was also higher than that estimated by Coulter Multisizer. Similarly, the size and bio-volume of *M. aeruginosa* determined by light microscopy were higher than that given by the Coulter Multisizer (see Table 2.3).

No changes in the size distribution pattern of *P. boryanum*, *M. aeruginosa* and *C. vulgaris* with time were observed following immersion in ISOTON II. (Fig. 2.7). However, in all instances, sample colony number and cell number at different durations of preservation were significantly different ($P < 0.01$) from fresh samples (Table 2.4).

Table 2.1 Channelizing count losses (%) and total count ml^{-1} ($x10^3$) at different range of counting rate.

Channelizing count loss (%)	Count s^{-1}	Total count ml^{-1} ± S.E.
1	200 - 300	91.58 ± 0.61
2	350 - 450	90.37 ± 0.47
3	500 - 600	89.43 ± 0.38
4	700 - 800	89.79 ± 0.83
6	900 - 1100	88.62 ± 0.64
7	1200 - 1400	88.51 ± 0.72
10	1500 - 1700	87.97 ± 0.86
12	1800 - 2000	87.81 ± 0.87
16	2200 - 2500	88.02 ± 0.91
22	2600 - 2800	87.94 ± 0.88

Table 2.2 Number of colonies or cells ml^{-1} (± S.E) obtained by different counting methods (n = 50 for all means).

Counting method	*Microcystis* count ml^{-1}	*Pediastrum* count ml^{-1}	Time required for 10 sample count with five replicate
Coulter Multisizer	78.34×10^5 (± 0.21)	102.62×10^3 (± 0.84)	50 minutes
Haemacytometer	77.53×10^5 (± 0.56)	-	140 minutes
Sedgewick rafter cell	-	97.26×10^3 (± 1.32)	175 minutes

Table 2.3 Equivalent spherical diameter (ESD), volume and size of algae measured by light microscope and Coulter Multisizer.

		Light microscopy	Coulter Counter
Pediastrum boryanum	ESD	-	59.07±7.08 µm
	Mean Volume	16015 µm³	15243 µm³
	Diameter	63.86±8.43 µm	-
Microcystis aeruginosa	ESD	-	4.13±1.03 µm
	Mean Volume	51.31 µm³	39.06 µm³
	length	8.0±2 µm	-
	width	4.0 µm	-

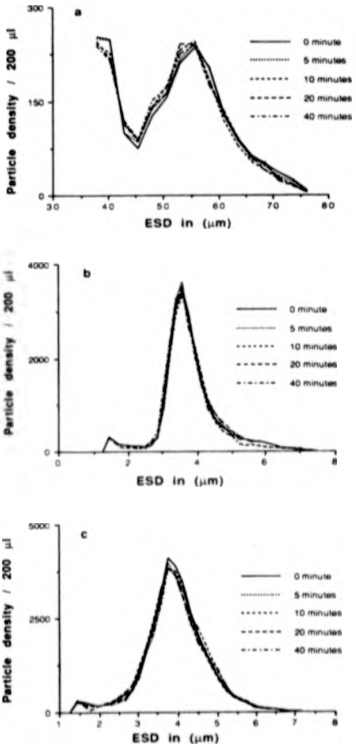


Fig. 2.7 Colony distribution pattern of (a) *P. boryanum*, (b) *M. aeruginosa* and (c) *C. vulgaris* following different durations in ISOTON II.

Reductions in average numbers of *P. boryanum* colonies were observed at all concentrations of preservative and in all durations of preservation studied (Fig.2.8a and Fig.2.9a). After 24 h, the average number of *P. boryanum* colonies was found to have decreased by 4.9 %, and after 70 days by 22.9% (Table 2.5). When mean colony sizes were compared, no significant shrinkage of *P. boryanum* colonies was observed during 24 h of preservation (Fig.2.10).

After 24 h, the mean number of *M. aeruginosa* colonies was found to have decreased by 2.7%, and after 70 days it had decreased by 29.4% (Table 2.5). The rate of disintegration of *M. aeruginosa* colonies appeared to decrease with time between 2 days and 70 days preservation (Table 2.5 and Fig. 2.8b). Some shrinkage of *M. aeruginosa* cells, as evidenced by a shift in peak distribution, was observed after 24 h preservation at all concentrations of Lugol's iodine (Fig. 2.11). Decreases in average colony numbers were observed to be significantly higher ($P < 0.05$) in 0.05% Lugol's iodine (Fig.2.9b).

By comparison, *C. vulgaris* cells were found to be less affected after 70 days. After 24 h and 70 days, the average number of *C. vulgaris* cells was found to have decreased by 4.7% and 14.8% respectively (Table 2.5). Shrinkage of *C. vulgaris* cells was also observed after 24 h preservation in all concentrations of Lugol's iodine (Fig. 2.12). No significant differences in decreases in average cell numbers were observed among the different concentrations of different preservatives (Fig. 2.9c)

Table 2.4 Results of analysis of variance for the effects of different concentrations of preservatives at different durations of preservation on (a) *P. boryanum*, (b) *M. aeruginosa*, (c) *C. vulgaris*

(a)

Sources of variation	d.f.	Sum of Squares	Means Squares	F-ratio	Sig.level
Concentration	7	378286	54041	310.58	P < .01
Duration	7	1902442	271777	1561.94	P < .01
Interaction	49	620175	12657	72.74	P < .01
Error	128	22215	174		
Total	191	2923119			

(b)

Sources of variation	d.f.	Sum of Squares	Means Squares	F-ratio	Sig.level
Concentration	7	1.426E+09	203699632	11486.39	P < .01
Duration	7	1.041E+09	148712768	8385.74	P < .01
Interaction	49	1.128E+09	23018000	1297.96	P < .01
Error	128	2269906	17734		
Total	191	3.597E+09			

(c)

Sources of Variation	d.f.	Sum of Squares	Means Squares	F-ratio	Sig.level
Concentration	7	31992602	4570372	267.45	P < .01
Duration	7	471607616	67372520	3942.45	P < .01
Interaction	49	8530856	174099	10.19	P < .01
Error	128	2187435	17089		
Total	191	514318496			

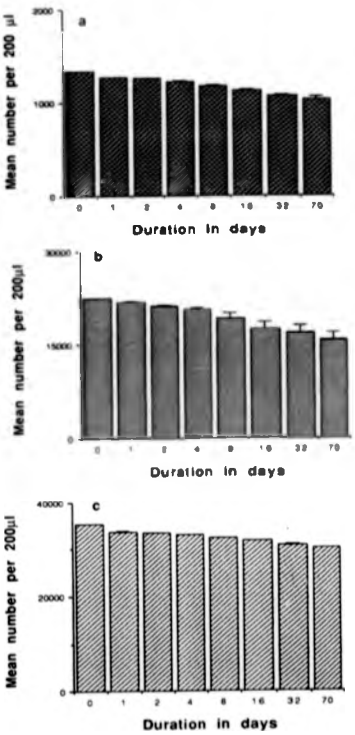


Fig. 2.8 Effects of different durations of preservation on the mean cell number for all concentration for both preservatives of (a) *P. boryanum* (b) *M. aeruginosa* and (c) *C. vulgaris*. Bars indicate ± S.E.

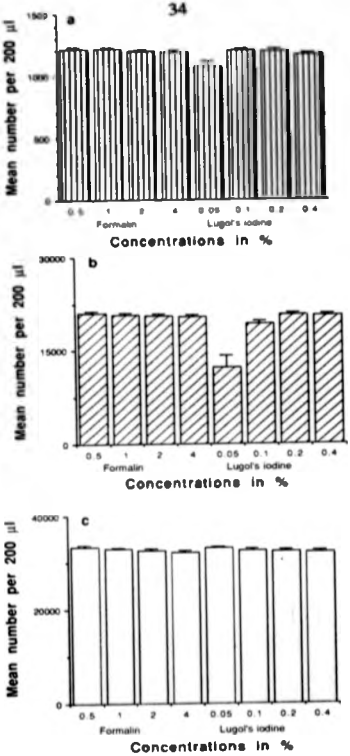


Fig. 2.9 Effects of different concentrations of both buffered formalin (column 1-4) and Lugol's iodine (column 5-8) on the mean cell number of all time series of (a) *P. boryanum* (b) *N. seruginosa* and (c) *C. vulgaris*. Bars indicate ± S.E.

Table 2.5 Mean percentage decrease in numbers of algal colonies and cells following different durations of preservation for both preservatives and all concentrations.

Algae	1 day	2 days	4 days	8 days	16 days	32 days	70 days
Pediastrum boryanum	4.94	5.92	8.32	12.29	15.97	20.39	22.86
Microcystis aeruginosa	2.67	5.33	7.82	14.30	21.91	24.78	29.36
Chlorella vulgaris	4.69	5.45	6.55	8.63	10.26	12.77	14.82

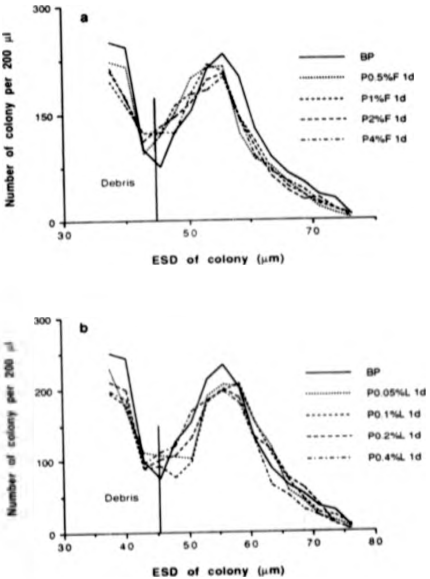


Fig. 2.10 Colony size distribution patterns of *P.boryanum* before preservation (BP) and after 24 hours preservation in (a) buffered formalin of concentrations 0.5%, 1%, 2%, and 4% (b) Lugol's iodine of concentrations 0.05%, 0.1%, 0.2% and 0.4%.

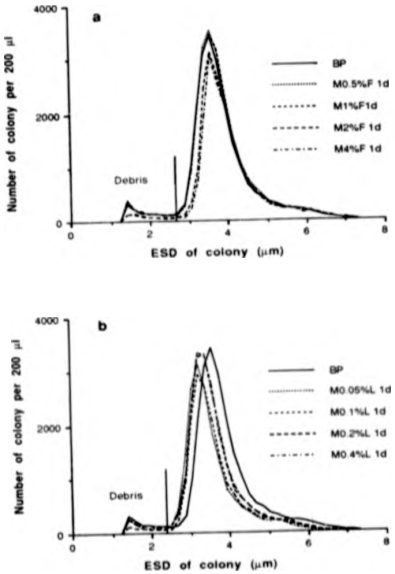


Fig. 2.11 Colony size distribution pattern of *M. aeruginosa* before preservation (BP) and after 24 hours preservation in (a) buffered formalin of concentrations 0.5%, 1%, 2% and 4% (b) Lugol's iodine of concentrations 0.05%, 0.1%, 0.2% and 0.4%.

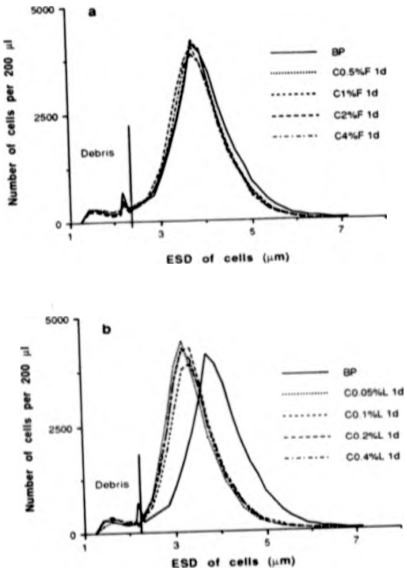


Fig. 2.12 Cell size distribution pattern of *C. vulgaris* before preservation (BP) and after 24 hours preservation in (a) buffered fomalin of concentrations 0.5%, 1%, 2%, and 4% (b) Lugol's iodine of concentrations 0.05%, 0.1%, 0.2% and 0.4%.

2.4 Discussion

Channelizing count loss increased with increasing counting rate (Table 2.1). Count loss by electronic particle counter was also reported by Kersting (1985) and as stated in Section 2.1, he recommended a rate of 200 counts/second. He suggested the count loss was due to dead time of the machine *i.e* the machine fails to count every particle. The channelizing count loss in this experiment may have been the result of this phenomenon. An alternative explanation is that there are errors inherent in the equipment in assigning particles to specific channels.

The Coulter Multisizer gave slightly higher, although not statistically significant, counts than the haemacytometer and significantly higher ($P < 0.05$) counts than the Sedgewick-rafter cell (Table 2.2). This may have been due to the instrument counting debris and other particles in addition to algal cells and colonies whereas the haemacytometer and Sedgewick-rafter cell counts were for algae alone. It was estimated in the present study that it required 40 minutes to count ten algal samples by Coulter Multisizer, whilst the same counts by haemacytometer and Sedgewick-Rafter cell required 120 minutes and 140 minutes respectively. Discussions with other workers using automated and manual mathods (Y. Waddell, personal communication) confirm that automated counting required significantly less time than manual counting. Moreover it is possible using the Coulter Multisizer to send data directly to a computer for storage and further statistical analysis, thus saving even greater amounts of time.

The ESD and volumes of *M. aeruginosa* and *P. boryanum* measured by microscope were significantly different from those obtained from the Coulter Multisizer. These differences are probably due to differences in algal cell size and shape. Vanderploeg (1981) reported that the Coulter counter significantly underestimated the size of long and thin algae. Bakker *et al.* (1985) also concluded that the Coulter counter may underestimate the volume and ESD of cylindrical, elongated or needle-like phytoplankton. The present findings further confirmed that electronic counting devices underestimate the bio-volume of long and thin algal cell and that their use in bio-volume estimation should be largely restricted to cells or colonies spherical in shape.

No changes in size distribution pattern of fresh *P. boryanum*, *M. aeruginosa* and *C. vulgaris* were observed following different durations of immersion in ISOTON II (Fig. 2.7). This is probably because the concentration of ISOTON II approximates that of the culture media and thus does not change the osmotic pressure inside the algal cell.

Visual observation of samples showed that after 8 days preservation in 0.05% Lugol's iodine, *P. boryanum* and *M. aeruginosa* samples were cloudy, possibly as a result of algal disintegration or possibly because of inadequate preservation and growth of microorganisms. Examination of samples by light microscopy confirmed that many cells had disintegrated.

P. boryanum and *M. aeruginosa* samples were more adversely affected by both Lugol's iodine and buffered formalin at all concentrations than *C. vulgaris* samples, possibly because of differences in cell wall structure. Breteler (1985) observed that no losses of marine diatoms occurred when preserved with Lugol's iodine, although flagellates were drastically affected after only 24 h preservation. Breteler (*ibid*) concluded that this difference was due to the diatom silica cell wall.

There is a clear indication in this study that colonial and cellular disintegration increases with preservation time. Published work, although scant (Weber 1968; Silver and Davoll 1978; Holtby and Knochel 1981; Paerl 1984; Breteler 1985), indicates that all commonly used algal preservatives or fixation methods, such as freeze-fixation, can damage algal cells. Indeed, the warning is often given that small, delicate cells may be destroyed by preservatives. In some instances, modified versions of preservatives may be suggested: for example, the replacement of acetic acid with sodium acetate is recommended for Coccolithophores, whilst for long-term storage of algae, it is recommended that the concentration of Lugol's iodine be increased and that formalin also be added (AHPA, 1989). In the light of the findings from the present study, it is recommended that more work on the development of algal preservatives be urgently carried out.

The advantages and disadvantages of the automated counting method, as described in the present study, can be summarised as follows. The Coulter Multisizer facilitates rapid counting. Moreover it gives additional information on size distribution patterns, equivalent spherical diameter (ESD), surface area and volume of particle. The Coulter Multisizer can send data directly to a computer, facilitating storage, analysis and presentation. Minor changes in the size distribution pattern can readily be detected by Coulter Multisizer which would be difficult by other methods. However, the Coulter Multisizer cannot readily distinguish between algae and other particles of the same size and it significantly underestimates the size and volume of long, thin algal cells. It is therefore best used with these disadvantages in mind and with routine comparisons with material examined by conventional light microscope.

CHAPTER III

Histology and Ultrastructure

3.1. Introduction

As plankton feeders rohu, catla and silver carp have comparatively well-developed gill rakers. Some anatomical and structural studies of gill rakers of silver and bighead carp have been made (Wilamovski, 1972; Iwata, 1976; Cremer and Smitherman, 1980; Jirasek, *et al.*, 1981;), most having been carried out on adult fishes. Spataru, *et al.*,(1983) reported that the average space between the gill rakers of silver carp and bighead carp of 15 - 30 cm standard length size was 36 µm and 84 µm respectively. Smith (1989) described the gill raker apparatus of silver carp (mean weight 32.5g) as a mechanical, passive sieve, which cannot filter particles of less then 10 µm, whereas Adamek and Spittler (1984) reported that silver carp (three years old, average weight 790g) can ingest particles of < 1 - 3.37 µm. By comparison, little work has been carried out on the gill rakers of catla and rohu.

The role of mucus cells in filter feeding has been suggested by many workers and might be an explanation of how particles smaller than the inter-gill raker spaces can be trapped. Greenwood (1953) described mucus entrapment of phytoplankton by

Oreochromis esculentus during filter feeding. Sinha and Moitra (1975) found many mucus cells associated with the pharyngeal and anterior oesophageal region of *Labeo rohita*, and suggested that they played an important role in trapping and transporting food particles during feeding. Sinha (1975) also observed the presence of large numbers of mucus cells in the buccopharyngeal region of *Catla catla* and *Cirrhina mrigala*. The presence of different types of mucus cells in the buccopharyngeal region of *Cyprinus carpio* has been reported by Sibbing and Uribe (1985). Mucus entrapment of food particles during suspension feeding in other fishes has also been suggested (Robotham, 1982; Beveridge et al., 1988; Northcott and Beveridge,1988; Sanderson, *et al.*,1991).

In many fishes, including cyprinids, food particles are ingested along with the water. There are a number of hypotheses concerning the selection of food particles from the water by fish. Sibbing and Uribe (1985) described the role of taste buds in the feeding of *Cyprinus carpio*. Sibbing (1987) reported that the taste buds in the pharyngeal roof of *Cyprinus carpio* lie on top of a muscular cushion, the palatal organ, a thick, protruded floppy structure suspended from the roof of the buccopharyngeal cavity, which is active in selection and food transport. Hlohowskyj *et al.* (1989) suggested that all seven species of the herbivorous cyprinid genus *Hybogmathus* bear taste buds or papillae in the pharyngeal region and that they are arranged in a pattern that helps in filtering or trapping diatoms and other small food items. Smith (1989) found that non-food particles would not induce feeding in silver carp, whereas organic matter from the

gravel filter, after being filtered through a 5 μm filter, induced vigorous feeding. Catla and silver carp also possess a palatal organ. In the case of rohu, the palatal organ is slightly different and resembles a muscular cushion provided with several honeycomb-like projections or papillae (Sinha and Moitra, 1975). Boulenger (1901) first described the base of this palatal organ in *Hypophthalmichthys* spp. as the suprabranchial organ. Wilamovski (1972) reported that the involvement of the suprabranchial organ in filter feeding of silver carp was as a pump. He also observed that each gill arch and its canal enters into a tube which extends into the palatal tissue block to form the hollow, spiral suprabranchial organ surrounded by the muscle tissue of the palatal organ. Sibbing and Uribe (1985) observed that the surface of the palatal organ of *Cyprinus carpio* is irregularly but heavily papillated and densely packed with taste buds on the tops of columnar-shaped papillae, even more dense in the lateral areas than in the medial region, and plays an important role in distinguishing food from non-food material during feeding. Sanderson *et al.*(1991) reported that the mucus-covered roof of the oral cavity plays an important role in the retention of particles in the suspension-feeding cyprinid blackfish, *Orthodon microlepidotus*.

A review of published literature fails to provide a clear picture of the filtering apparatus and its associated organs in rohu, catla and silver carp, particularly the fry and fingerling stages. Most published studies to date have been limited to gill raker morphometry and histology of the alimentary canal of adult fish. In view of this it was decided to study the bucco-pharyngeal region and the filtering apparatus associated

with the filter feeding of *Labeo rohita*, *Catla catla* and *Hypophthalmichthys molitrix*. The study included detailed ultrastructural and histological investigations to elucidate possible feeding mechanisms.

3.2 Materials and Methods

3.2.1 Fish

Two-day old fry of *Labeo rohita*, *Catla catla* and *Hypophthalmichthys molitrix* were imported from the Fisheries Research Institute, Mymensingh, Bangladesh in sealed plastic bags. They were held initially in a static water quarantine system with aeration for 4 weeks until preliminary disease monitoring could be completed. They were then transferred to a recirculatory system for rearing. They were fed on a mixed diet composed of algae, Artemia and ground trout pellets. Fish of standard length (SL) 30 - 92 mm were used in the experiments.

3.2.2 Feeding apparatus morphometrics

The buccopharyngeal feeding structures, (*i.e.* gill arches) were carefully removed from the fish with the aid of a binocular dissecting microscope (Olympus VM). All specimens used in this study had been previously preserved in 10% neutral-buffered formalin. Preliminary examination of the gill apparatus showed a considerable degree

of variation among the gill arches. In order to standardize observational data gill arch II was chosen for morphometric study. Once it had been excised and prepared for morphometric data the following measurements were made using an Olympus BH2 light microscope :

(a) gill arch length (from the base of the anterior most gill raker to the base of the posterior most gill raker);

(b) the number of gill rakers;

(c) gill raker length;

(d) the distance between the gill rakers.

At least 10 measurements for (c) and (d) were made from the mid-section of gill arch II. The lengths and distances between gill rakers from the mid-section of gill arch II were used to determine the relationship between the standard length and the chosen gill raker data from the fish.

In order to obtain detailed descriptions of the feeding apparatus and for a comparative study among rohu, catla and silver carp the following data were taken from fishes of approximately 90 mm standard length:

(a) length of each gill arch;

(b) number of gill rakers on each gill arch;

(c) length of all individual gill rakers on each gill arch;

(d) taste bud number and spacing on the gill raker in the mid-section of gill arch II;

(e) number of taste buds per mm^2 on the roof of the buccopharyngeal cavity.

Counting of taste buds on the gill raker and roof of the buccopharyngeal cavity was carried out by both electron and light microscopy.

The area of the filtering part of individual gill arches was determined using the following formula developed from Jirasek *et al.* (1982) and Gibson (1988):

$$Fa = (L \times l) \times 2$$

where Fa = filtering part of the gill arch; L = length of the gill arch; l = average length of the pharyngeal surface of gill raker.

Fifteen fishes for each species were used in this purpose ranging from 30 to 100 mm Standard Length (S.L.).

3.2.3 Scanning electron microscopy (SEM)

For scanning electron microscopy (SEM) silver carp, rohu and catla of similar size were selected and deheaded after being killed by an overdose of 10% benzocaine (Ross and Geddes, 1979). A standard method was used for the processing of specimens (Reutter *et al.*, 1974; Sibbing and Uribe 1985). The specimens (heads) were washed in buffered physiological saline to remove mucus and fixed for seven days at 4°C in 3% glutaraldehyde, buffered at pH 7.2 in 0.1 M sodiumcacodylate containing 2.5% glucose. The heads were then sectioned horizontally into a dorsal and ventral half to investigate the floor and roof of the buccopharyngeal region and intact gill arches carefully removed for detailed study. Specimens were then washed repeatedly in a buffered saline solution prior to being transferred to 1% osmium tetroxide, buffered in 0.1 M sodiumcacodylate, for 6 h for final fixation. After repeated washing in the buffer the specimens were then dehydrated through a graded acetone series, stored in 70% acetone if necessary, and critical point dried in a Polaron E-1000 dryer before being mounted on aluminium stubs with silver conducting paint and coated with a layer of gold in an Edwards S150B sputter coater. A Phillips 500 Scanning Electron Microscope was used for examination of the specimens.

3.2.4 Histology

Specimens from *L. rohita*, *C. catla* and *H. molitrix* were fixed in 10% neutral buffered formalin for at least a week and then processed by automatic tissue processor and embedded in wax (Appendix - 8). A series of sections was made after trimming and decalcification (4h in 15% HCl)of the specimen. All sections were 5 µm in thickness.

Serial sections were stained with Haematoxylin-Eosin (H&E) for structural analysis. Neutral as well as acid mucus is stained with Periodic Acid Schiffs reagents (P.A.S) whereas Alcian Blue (AB) at pH 2.5 was used for acid mucopolysaccharides and glycoproteins (Drury and Wallington, 1980). All sections were examined using a light microscope fitted with an eyepiece graticule.

An accurate assessment of the density of the mucus cells was not attempted. However, the densities of different types of mucus cells in different areas were recorded for comparative purpose.

3.3 Results

3.3.1 Introduction: terminology and subdivisions of the oro-pharynx

Using conventional terminology the oral cavity is assumed to extend from the lips to

the level of the lower jaw. The buccal cavity extends from here to the base of the first branchial arches (Fig 3.1.). The pharyngeal cavity in cyprinids is sub-divided into the anterior pharynx (syn. branchial cavity), containing the gill arches and is involved in respiration and food selection, and the posterior pharynx (syn. chewing cavity). The respiratory filaments of the gill arches project into the opercular cavity.

3.3.2 *Labeo rohita*

Oro-buccal cavity

Lips: The toothless ventral mouth is bordered by thick soft upper and folded lower lips, the upper lip overlapping the lower lip. The lips are fringed and bear large numbers of taste buds (240/mm^2) (Plate 1a) on the tops of closely-packed epithelial folds. Taste buds are absent at the mouth angles.

Oro-buccal roof: A smooth, thin, crescent-shaped respiratory valve projects from the oral roof at the inner edge of the upper jaw (Plate 1a). No taste buds were found on

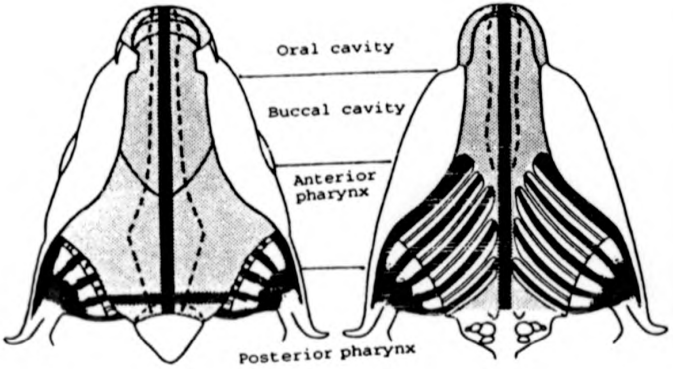


Fig. 3.1 Transverse section of skull showing the sub-division of oro-pharyngeal region.

the distal half of the valve whereas some papillae with taste buds were found on the basal part (40/mm^2).

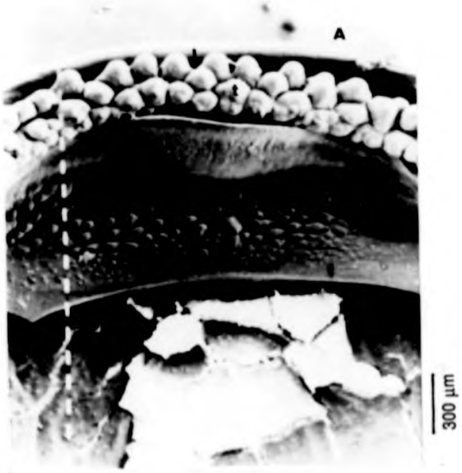
Oro-buccal floor: Small papillae were found to extend inwards from the horny area of the lower jaw. Longitudinal folds were observed which run caudo-laterally towards the first branchial slit (Plate 2a). These folds allow considerable extension of the buccal walls. A few taste buds (25/mm^2) were found, and only at the top of these folds. There is no true tongue although the central convex area of the buccal floor which is often referred to as a tongue is supported by the mobile glossohyal arch which is connected to the hyoid arch. Many papillae with taste buds (65/mm^2) were present in this area (Plate 2a).

Pharyngeal cavity

Anterior pharynx: The roof of the anterior pharynx is formed by a muscular cushion, the palatal organ, covering the base of the skull. The surface of the palatal organ closely fits the pharyngeal surface and curvature of the gill arches. The surface of the palatal organ is a honeycomb-like projection and is densely packed with taste buds (Plate 3a). The density of taste buds in the medial region is less (average up to 170 /mm^2) than the lateral area (average up to 320 /mm^2). In the posterior region of the palatal organ the number of taste buds is markedly reduced (average up to 130 /mm^2).

Plate 1. SEM veiw of lips and respiratory valve (a) rohu, (b) catla, (c) silver carp and (d) closer veiw of the base of the respiratory valve of catla as marked by d in figure b.

a



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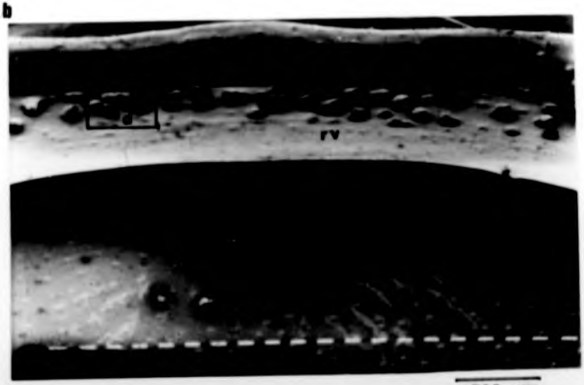
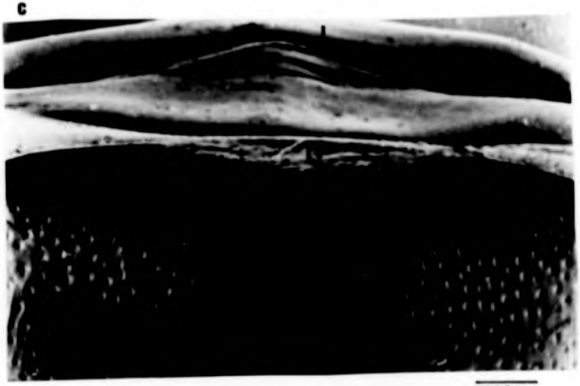
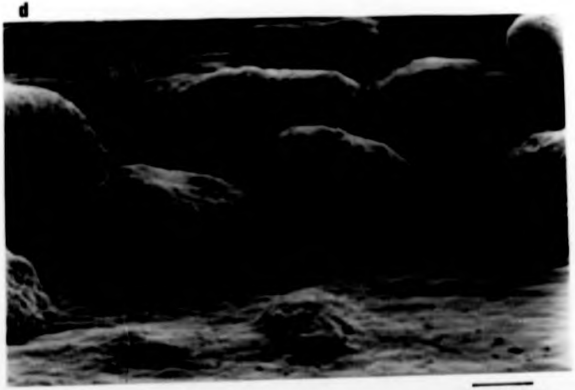


c



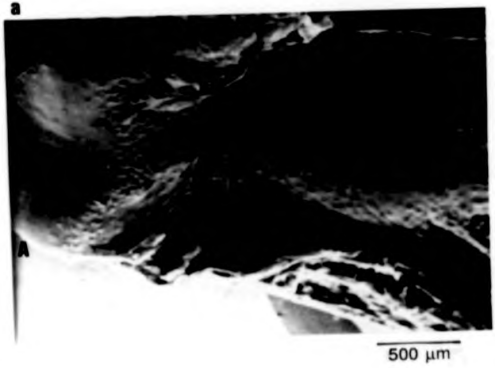
300 µm

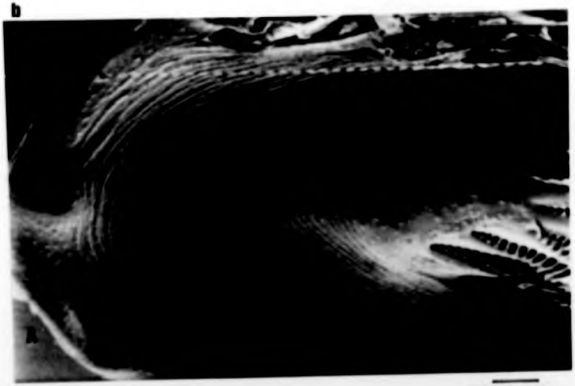
d



50 µm

Plate 2. SEM survey of buccal floor (a) rohu, (b) catla and (c) silver carp.


a
A
500 µm

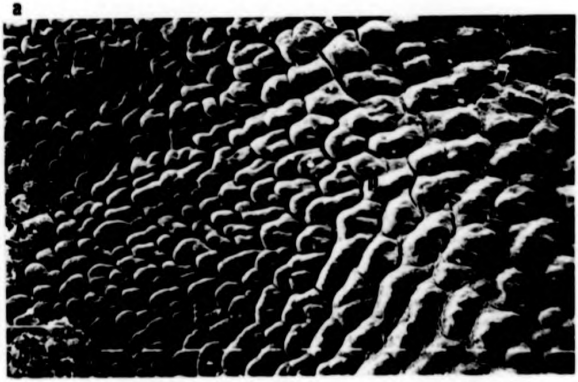

b
A
500 µm

c

500 µm

Plate 3. SEM study of Pharyngeal roof (a) rohu, (b) catla and (c) silver carp.

a



200 µm

b

400 µm

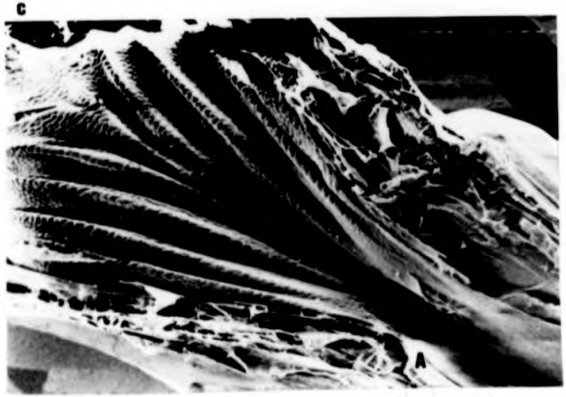
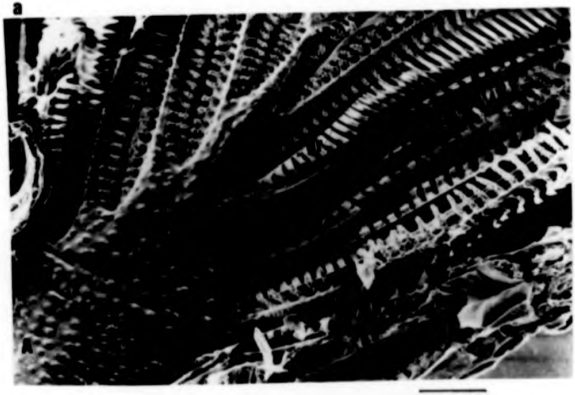

C
A
500 µm

Plate 4. SEM veiw of the pharyngeal floor (a) rohu, (b) catla and (c) silver carp.

a



400 µm

b

400 µm

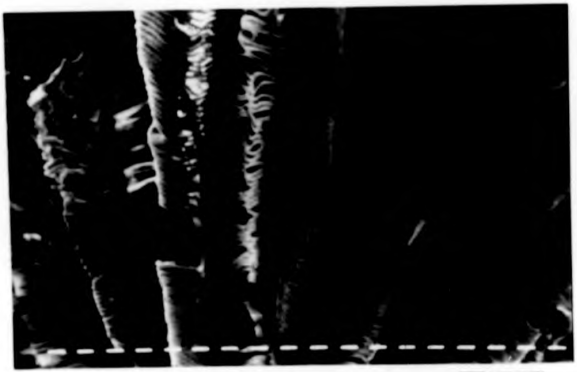
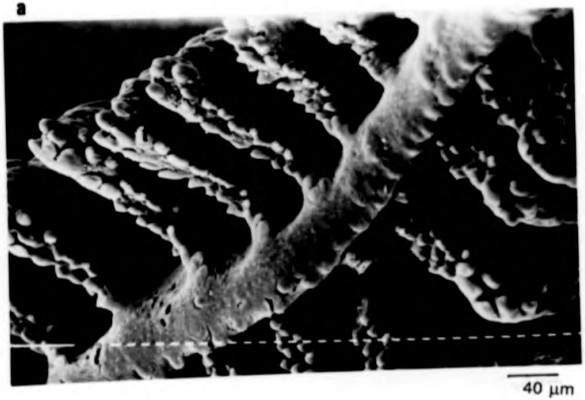
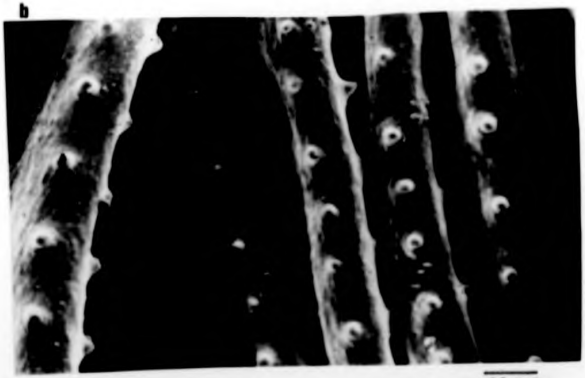

400 µm

Plate 5. SEM survey of pharyngeal surface of gill rakers (a) rohu, (b) catla and (c) silver carp

a



b




c
20 µmm

The floor of the anterior pharynx (Plate 4a) is largely composed of the gill arches and associated rakers. The gill arches and the double rows of rakers form a perforated plane along the floor of the pharyngeal cavity. The pharyngeal surfaces of the gill rakers bear numerous small projections or conical papillae with taste buds (Plate 5a). Two rows of gill rakers are attached by a thin muscular strip of tissue along the gill arches and the pharyngeal surface of this tissue bears very few taste buds. The gill-rakers from subsequent arches are arranged in such a way that they can be useds for food selection.

Gill raker length (GRL) and gill raker space (GRS) were observed to increase with increasing fish standard length (SL) and the relationship between fish standard length and gill raker length; and fish standard length and gill raker space are shown in Fig. 3.2 and may be described by the equations:

$$GRL = -1634.5 + 1261.3 \log_e SL;\ r = 0.98$$

$$GRS = -8.10 + 53.36 \log_e SL;\ r = 0.97$$

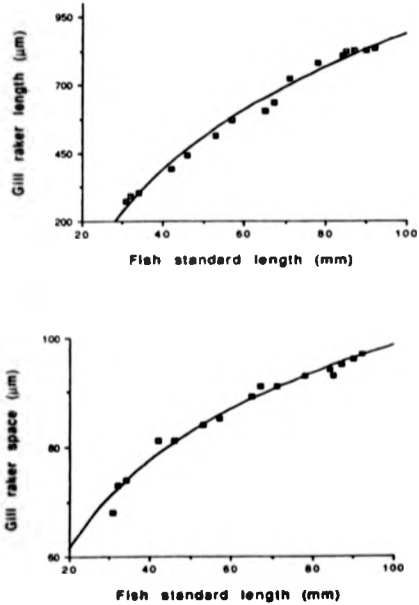


Fig. 3.2 The relationships between (a) gill raker length, (b gill raker space and fish standard length of rohu.

3.3.3 *Catla catla*

Oro-buccal cavity

Lip: The upturned protrusile, toothless mouth is bordered by thick folded lips with taste buds (40/mm^2) along the inner surface (Plate 1b), whereas the folded part is almost devoid of taste buds. No taste buds are found at the mouth angles.

Oro-buccal roof: A crescent-shaped respiratory valve projects from the oral roof at the inner edge of the upper jaw (Plate 1b). No taste buds were found on the respiratory valve other than few at the base (40 taste buds/ mm^2). At the base of the respiratory valve several oval-shaped papillae with taste buds are present (Plate 1d). The roof of the oral cavity bears small papillae with taste buds (110/mm^2).

Oro-buccal floor: No mandibular valve is present. Large numbers of longitudinal folds bear papillae and taste buds at the top of the folds which run caudo-laterally towards the first branchial slit (Plate 2b). There is no true tongue, although the central convex area of the buccal floor, which looks like a tongue, is supported by the mobile glossohyal, connected to the ventral elements of the hyoid arch. Medium-sized papillae with taste buds are universally conspicuous in this area (110/mm^2).

Pharyngeal cavity

Anterior pharynx: The roof of the anterior pharynx is formed by a bi-lobed palatal organ. The surface of the palatal organ closely fits with the pharyngeal surface of the gill rakers.

The surface of the bi-lobed palatal organ has three ridges at each side which are heavily papillated (Plate 3b) and densely packed with taste buds (270/mm^2). In between the ridges are found few papillae with low densities of taste buds (90/mm^2). In the posterior part of the palatal organ, the triangular area enclosed between the gill arches and the chewing pad, the papillae are densely packed although there are markedly reduced numbers of taste buds (90/mm^2).

The floor of the anterior pharynx (Plate 4b) is mainly composed of the gill arches and associated rakers. The gill arches and double rows of rakers form a perforated bi-lobed plane with four canals at each side that lie immediately adjacent to the palatal organ when the floor of the mouth is raised.

The pharyngeal surface of the gill rakers bears two rows of small papillae, each with a single terminal taste bud (Plate 5b). The gill rakers are not fixed, and may be adjusted during food selection.

Gill raker length (GRL) and gill raker space (GRS) were also observed to increase with standard length (SL). The relationship between fish standard length and gill raker length and fish standard length and gill raker space are shown in Fig. 3.3 and can be described by the following equations:

$$GRL = -2221.0 + 1969.1 \log_{10} SL;\ r = 0.96$$

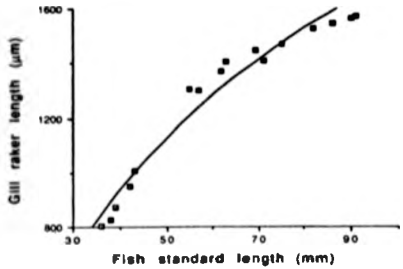
$$GRS = 27.32 + 52.64 \log_{10} SL;\ r = 0.97$$

3.3.4 *Hypophthalmichthys molitrix*

Oro-buccal cavity

Lips: The protrusile toothless mouth is bordered by thick lips with a horny edge. The middle of the lower lip has a projection (Plate 2c).

Oro-buccal roof: A crescent-shaped respiratory valve projects from the oral roof at the inner edge of the upper jaw (Plate 1c). The distal part of the valve is smooth and taste buds are almost completely absent, whereas the base of the valve is papillated with taste buds (120/mm^2).



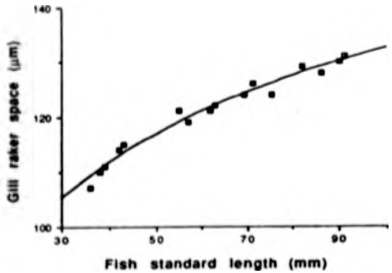


Fig. 3.3 The relationships between (a) gill raker length, (b gill raker space and fish standard length of catla.

Oro-buccal floor: Papillae similar to those on the surface of the oro-buccal roof extend inwards from the base of lower jaw. The density of taste buds is similar to that of the oro- buccal roof. There are some longitudinal folds at the level of the cheeks which are deep and run in a caudo-lateral direction towards the first branchial slit (Plate 2c). These folds allow considerable extension of the buccal walls. The central area of the buccal floor, often referred to as the tongue, is supported by the mobile glossohyal, connected to the ventral elements of the hyoid arch. Many papillae with taste buds are conspicuous throughout this area.

Pharyngeal cavity

Anterior pharynx: The roof of the anterior pharynx is covered with a bi-lobed palatal organ. The surface of the palatal organ closely fits with the pharyngeal surface of the gill rakers. In fresh preparations the palatal organ appears as a muscular cushion suspended from the pharyngeal roof. The muscular cushion contains the hollow spiral organs, referred to as the suprabranchial organ in the literature (Wilamovski, 1972).

The surface of the palatal organ has four prominent ridges on each side (Plate 3c) which are heavily papillated and densely packed with taste buds (530/mm^2). The density of taste buds between ridges is low (220/ mm^2), although there are many mucus cell openings. In the posterior part of the suprabranchial organ, the triangular

area close to the chewing pad is heavily papillated although with reduced numbers of taste buds (180/mm^2).

The floor of the anterior pharynx is composed of the gill arches and their rakers (Plate 4c). Each gill arch forms a perforated canal with its double row of gill rakers. The bi-lobed pharyngeal floor is formed by four perforated canals at each side and fits closely with the ridged roof of this area.

The pharyngeal surfaces of the gill rakers bear two rows of conical papillae with taste buds (Plate 5c). Gill rakers were observed to be fixed due to the fusion of parts of adjacent gill rakers as described by Iwata (1976). No such fusion of gill rakers was observed among fish below 30 mm SL. Small fish are thus able to move adjacent gill arches to collect food particles.

Inter-gill raker spaces were found to be almost constant (25-30 µm) irrespective of fish size, whereas gill raker length (GRL) was found to increase with increasing fish standard length (SL). The relationship between gill raker length and fish standard length is shown in Fig. 3.4 and by the equation:

$$GRL = -2098.6 + 1850.5 \log_e SL;\ r = 0.98$$

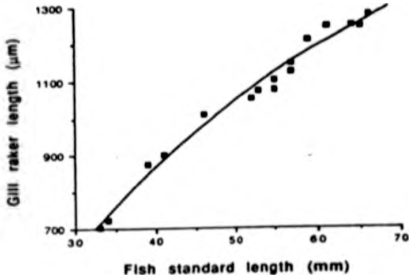


Fig. 3.4 The relationship between gill raker length and fish standard length of silver carp.

Suprabranchial organ: This is a specialized organ, which appears to hang from the roof of the pharyngeal surface, and is attached to the base of the gill arches. Each gill arch and its canal enters into a hollow tube lined with epithelial tissue bearing lots of mucus cells, and continues into the palatal tissue block to form the hollow spiral suprabranchial organ (Plate 6a, 6b).

3.3.5 Light and scanning electron microscopy of the oro-pharyngeal wall

Tissue layers

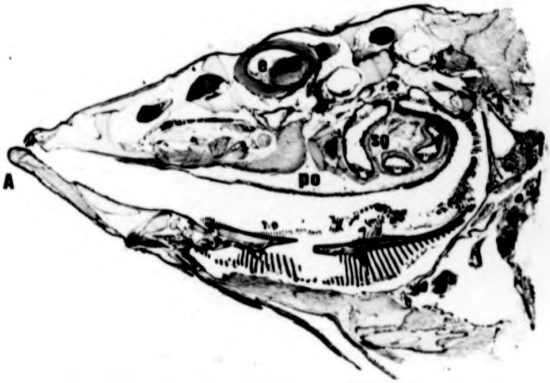
In general, the oro-buccal wall is covered by a stratified epithelium (25-90 μm thick). The underlying connective tissue stroma consists of:

i) stratum compactum, a compact layer of collagen fibers, 3-4 μm thick;
ii) a less dense fibrous layer, 8-20 μm thick, with interposed nucleated fibroblasts;
iii) a loose areolar connective tissue layer with blood vessels and nerves.

Palatal organ

The palatal organ has a covering of mucus epithelium (Plate 7). Taste buds lie along the flat top of the papillae. Mucus cells are dominant along the margins of adjacent

Plate 6. Histological section of suprabranchial organ of silver carp (a) transverse section (b) cross section.



b

papillae. The underlying fibres form muscular cores for the papillae and supporting platforms for the taste buds. The thick muscular tissue backing the papillae is composed of roughly longitudinally and transversely-oriented striated fibers.

3.3.6 Specializations of the oro-pharyngeal epithelium

The stratified oro-pharyngeal epithelium contains a number of specialized cell types including horny cells, mucus cells, club cells, chloride cells, sensory cells and round cells typical of fish epidermis (Whitear, 1971; Sibbing and Uribe, 1985). Their abundance varies locally and they serve a multitude of functions. The surface of the common epithelial cells is commonly intricately sculptured by microridges (Hawkes, 1974; Hughes, 1979; Ono, 1980; Sibbing and Uribe, 1985). In all the carps studied the microridges had a similar fingerprint-type pattern (Plate 8a, 8b and 8c) with minor variations.

In live fishes the whole epithelial surface is covered by a slimy, fibrous layer, approximately 1 µm thick, which is secreted from the underlying epithelial cells as demonstrated by Whitear (1970) in *Phoxinus* and a number of other teleost fishes. A similar external coating appears to cover the aperture of the taste buds of the bucco-pharyngeal region and is probably composed of glycoproteins. The mucus layer of the bucco-pharyngeal surface was lost during preparation of histological sections and during preparation of the SEM sample by washing specimens with buffered saline.

Plate 7. Transverse section of palatal organ x 600.

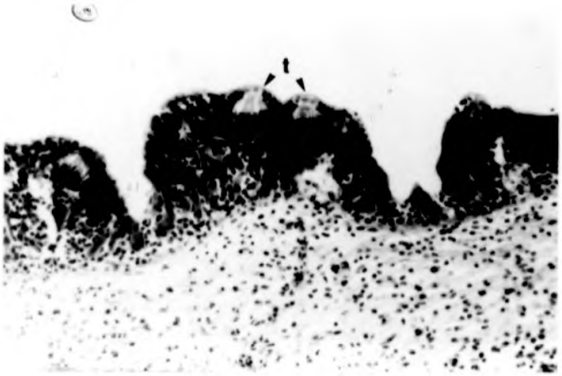
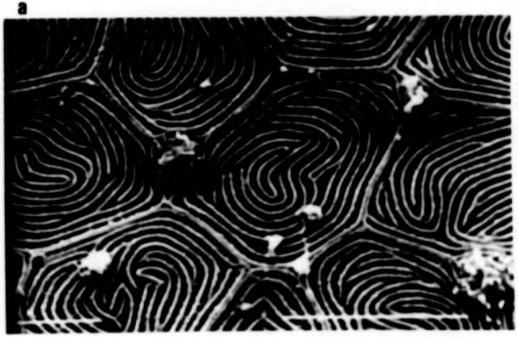
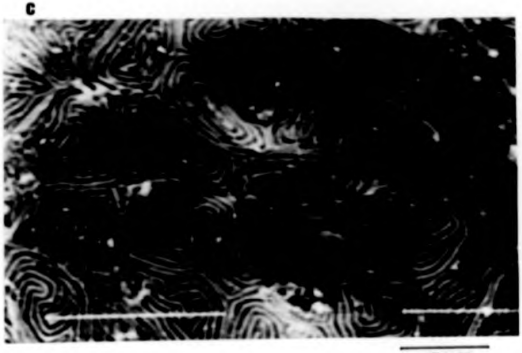


Plate 8. SEM survey of microridges at the bucco-pharyngeal region (a) rohu, (b) catla and (c) silver carp.

a



b


c
5 µm

Mucus cells

Mucus cells, usually referred to as goblet cells, are the most common unicellular glands of the fish epidermis (Henrikson and Matoltsy, 1968). According to differences in size and shape of mucus cells, it is possible to make a distinction between large clavate mucus cells and small goblet mucus cells.

The orobuccal surface of rohu, catla and silver carps consists largely of small goblet cells, which produce small quantities of mucus, mainly sialomucines. Few mucus cells were found on the lips and the anterior part of mouth in the carps studied. In the pharyngeal roof both the small goblet and large clavate cells were present, goblet cells containing sialomucines in the anterior, widest area of the palatal organ. In the posterior region numerous large sulfomucine-containing clavate cells are present. The distribution pattern of mucus cells was observed to be the same in all carps examined.

In the gill raker region of rohu and catla some large clavate cells are present. Silver carp, however, have no clavate mucus cells present in the gill-raker region.

Taste buds

Taste buds are pear-shaped sensory organs sometimes extending from the basal membrane to the free surface of the epithelium. Light and dark sensory cells run

through their length and taper towards the free apical end into a pore (Plate 9a). The light- coloured receptor cells bear one or two long apical processes, the dark cells having a large number of small microvilli. Only recently (Reutter, 1992) has it been reported that the dark cells are also sensory in nature instead of merely providing a supporting role as had been previously assumed (Hirata, 1966).

Taste buds in the carps studied differed somewhat in size (Table- 3.1) although the size differences were not significant ($P > 0.05$). The exposed receptor area of the taste buds measures 5.5- 12.5 µm in diameter.

Rohu were observed to bear large numbers of taste buds on their lips, compared with catla which had few taste buds on the lip surface, and silver carp which had no taste buds apparent on their lips. The density of taste buds over the oro-pharyngeal surface varied, highest densities being on the palatal organ and gill rakers of all species studied. Among the carps, however, silver carp had highest densities (530/mm^2) of taste buds on its palatal organ, followed by rohu (320/mm^2) and catla (270/mm^2). Catla and silver carp were also observed to bear two rows of taste buds on the pharyngeal surface of each gill raker (Plate 5b and 5c), whereas rohu bore taste buds only along the free top edge of the gill rakers (Plate 5a).

Table 3.1 Comparative data on taste bud density (No./mm^2) in different oro-pharyngeal regions and taste bud size (μm) ± S.D. of rohu, catla and silver carp of the same size (90 mm)

	Rohu	Catla	Silver carp
Lips	240	40	0
Oro-buccal roof	40	70	120
Oro-buccal floor	40	110	120
Anterior pharyngeal roof	250	180	370
Posterio pharyngeal roof	130	90	180
Pharyngeal floor	110	150	320
Taste bud size (exposed receptor diameter)	9.1 ± 2.0	8.8 ± 1.7	8.2 ± 1.7

3.4 Discussion

This study provides a description of the surface structure and histological information on the bucco-pharyngeal region of three of the most important filter feeding carps, rohu, catla and silver carp, during the fry and fingerling stages. The distribution pattern of mucus cells and taste buds provides some insight into the feeding and food selection mechanisms.

Filter feeding fish engulf food particles together with water and then collect it by sieving through the gill rakers. The roles of lips, the oro-pharyngeal surfaces, gill rakers and opercula in the feeding of fish have been well documented (Sinha and Moitra, 1975; Sibbing, 1982; Sibbing and Uribe, 1985; Northcott and Beveridge, 1988; Sanderson et al., 1991; Agrawal and Mittal, 1991 and 1992)

The lips of rohu are densely packed with taste buds whereas catla and silver carp were observed to have few taste buds on their lips. Sinha and Moitra (1975) stated that the taste buds on the lips of rohu helped in the search for suitable food from the water column as well as from surfaces. Taste buds on the lips may help the catla and silver carp to detect food particles in the water column. Agrawal and Mittal (1991) also suggest that the taste buds on the lips of catla help in searching for food particles during feeding.

Gill rakers were observed to have conical papillae with taste buds on their pharyngeal surface. The appearance of papillae with taste buds on the gill rakers of fish has been described by various authors, including Iwai (1964); Reutter et al. (1974); Hossler and Merchant (1983); Sibbing and Uribe (1985); Sibbing (1986); Northcott and Beveridge (1988). Fish may use the conical papillae on the gill rakers to reduce inter-raker space by interlocking in order to retain food particles, and utilize the taste buds in selecting food from non-food items.

Most studies of filter feeding mechanisms in fish have assumed a method of mechanical sieving by the gill rakers in which the gill raker apparatus functions merely as a passive sieve. Measurement of gill raker length and inter-gill raker space in determining the efficiency of filtration has been performed by King and MacLeod (1976), Durbin (1979), Spataru et al. (1983), Wright et al. (1983), Gibson (1988), Smith (1989), MacNeill and Brandt (1990), Berg et al. (1992).

Gill raker length and inter-raker space among the carps studied indicate that they are suited for filter feeding. The ratio of inter-raker distances in rohu, catla and silver carp is 1 : 1.37 : 0.32 respectively, suggesting that catla have the largest mesh size (lowest retention ability), silver carp the smallest mesh size (highest retention ability) and that rohu are intermediate. In passive sieving terms silver carp would thus appear to be a more efficient filter feeder than either rohu or catla. In the natural environment with smaller food particles silver carp may be able to collect the bulk of their food by

passive means. Adamek and Spittler (1984) found that silver carp are able to feed on particles less than 5 μm in size. By contrast, the gill raker spacings in rohu and catla suggest that they are unable to feed on small particles if passive mechanical sieving is the only means of filtration.

The capacity of a filter, in terms of volume filtered per unit time, is an important factor in determining the amount of food particles that can be retained per unit time. The capacity of a filter is determined by the amount of water passed through the filter and the area of the filter through which water flows (the filtering area). Using the modified version of the formula given by Gibson (1988) Fa = (L x l) x 2 (see above) in which the size of the gill raker elements and inter-gill raker spacing are both considered in estimating the total filtering area, it was found that the ratio among the three species was 1 : 2.78 : 3 for rohu : catla : silver carp. It suggests that silver carp have a considerably larger filtering area than the other species. This point will be considered further in Chapter VI.

The involvement of the pharyngeal roof of fish in food selection has been reported by Sibbing and Uribe (1985), Sibbing (1987), Hlohowskyj et al. (1989) and Sanderson et al. (1991). From this study it is clear that rohu, catla and silver carp have large numbers of taste buds (180-570/mm^2) in the pharyngeal roof area which may help them to detect and select food particles. In live carps the pharyngeal roof is closely fitted to the pharyngeal floor and both surfaces are densely packed with taste buds which

probably help the fish assess the shape and size of the food particle as well as determine its olfactory properties. In another experiment it was found that silver carp are capable of distinguishing toxic from non-toxic algae in suspension based on cell surface properties (see chapter IV). McGlone (1978) reported that the palatal organ of goldfish responds to tactile stimuli at all stages of denervation, with local bulging, possibly caused by proprioceptive reflex loops. Konishi and Zotterman (1963) found some glossopharyngeal nerve fibers in the palatal organ of the carp reacting exclusively to mechanical stimulation. Sibbing (1987) stated that common carp can isolate and select food particles from non-food particles with the help of taste buds on the pharyngeal surface.

Among the carps studied silver carp are unique in possessing a suprabranchial organ on the roof of the pharyngeal cavity. Wilamovski (1972) stated that the suprabranchial organ in silver carp works as a simple pump. It draws water into itself by expansion and accumulates food masses towards the back of the organ. It then contracts and pumps out the water, washing accumulated food masses through the gill raker mesh to the floor of the pharynx and the opening of the gullet. This study appears to confirm that the suprabranchial organ probably works as a pump and in addition, may help in collection of small food particles by entrapment by mucus cells.

The chemical properties of mucus were reviewed by Hunt (1970).Two main components are distinct, glycoproteins and mucopolysaccharides. Mucous composed

mainly of glycoproteins is referred to as sialomucine, while mucous consisting principally of mucopolysaccharides is known as sulfomucine.

Mucus performs a multitude of roles in a wide spectrum of biological systems (Whitear, 1970; Mittal and Banerjee, 1980; Cook and Shirbate, 1983; Sibbing and Uribe 1985; Northcott and Beveridge 1988), including (i) the formation of a mechanical barrier to foreign bodies and pathogens (it has no antibiotic effect in carp (Hattingh and Van Warmelo, 1975)); (ii) the formation of a chemical barrier which supports osmoregulation and prevents a flux of material over steep chemical or electrical gradients; (iii) the reduction of friction; (iv) the provision of a mechanical buffer which protects the epithelium from damage and abrasion; (v) aiding of precipitation of suspended mud by entrapment and cleaning of the epithelium (often by cilia); (vi) sticking together particulate food; (vii) prevention of desiccation by binding of water; (viii) the enhancement of adhesion in sluggish locomotion; (ix) serving a means of communication and navigation (e.g. in molluscs); (x) the feeding and attachment of young and (xi) formation of cocoons and other envelopes for temporary shelter.

The oro-buccal wall produces mainly sialomucines. This type of mucus is common in the respiratory and alimentary tract of vertebrates, probably serving principally as protection and lubrication (Hunt, 1970). Sialomucines may thus well aid in orobuccal water transport, especially at high suction velocities, when the energy costs increase

exponentially.

Observations on the origin, development and occurrence of mucus cells in the bucco-pharyngeal region of *Labeo rohita, Catla catla* and some other fishes have been made (Sarbahi, 1939; Moitra and Sinha, 1972; Sinha and Moitra, 1973, 1975; Sinha, 1975). Sibbing and Uribe (1985) found that mucus cells producing sialomucines occurred in the anterior part of the oro-pharynx of the carp and that the sulfomucines only appeared in the posterior part of the pharynx. Northcott and Beveridge (1988) also found two types of mucus cells in the gill rakers and bucco-pharyngeal region of *O. niloticus*

In this study sulfomucines were found in the posterior pharynx of all carps studied and in the gill raker epithelia of rohu and catla. This high viscosity mucus may help in trapping and aggregation of small food particles into boluses and may also assist in the transportation of food particles.

The low-viscosity sialomucines in the orobuccal cavity and anterior part of the pharynx may serve as lubricants in reducing friction due to water currents during feeding and protecting the epithelium from damage.

No work has yet been carried out to ascertain the physical properties and secretion kinetics of oro-pharyngeal mucus in fish. It is expected that the secretion of mucus is

somehow regulated in accordance with the feeding activities of the fish.

Mechano- and chemoreceptors are required to detect the qualities of the ingested food material during subsequent food processing. Moreover fish also need to be able to constantly monitor the quality and velocity of water during respiration and feeding.

Reutter et al., (1974) distinguished three types of taste buds in the headgut of the sword-tail (*Xiphophorus helleri* Heckel) based on their position with respect to the surrounding epithelium. Accepting Reutter's theory, taste buds protruding most (Type I), which would be most effective in mechanoreception, occur in the gill rakers of rohu, catla and silver carp, in the lips of rohu and in the buccopharyngeal regions of all species (Plate 9a). Less protruding taste buds (Type II) line the epithelial surface (Plate 9b). Type III taste buds never rise above the normal level of the epithelium (Plate 8c). The other type which was found in the oro-buccal wall in silver carp (Plate 9c), is similar in structure to those found on the inner surface of the carp operculum and which was suggested as serving a chemoreceptor function (Sibbing and Uribe, 1985).

During filter feeding fish may need information about size, density and olfactory properties of food particles. Experiments in Chapter IV indicate that the density and size of algae and the toxic and non-toxic properties of the algae influence the fish's decision to filter feed. It is proposed that the high density of taste buds on the gill

Plate 9. SEM study of taste buds (a) type I, (b) type II and (c) type IV.

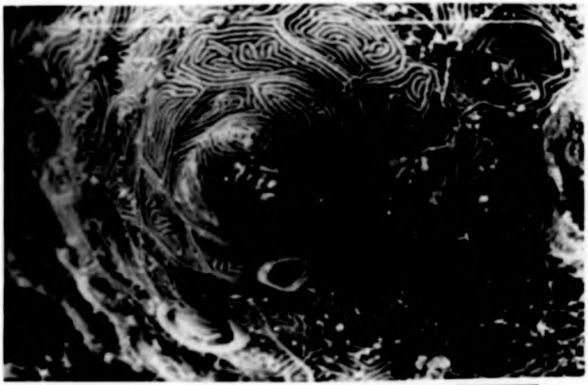
a

2 μm

b

2 μm

c



rakers and other oro-pharyngeal regions assist the fish in obtaining information about potential food particles. This information can then be used to decide whether or not to ingest the particles and whether, if possible, any adjusment of the filtering apparatus is necessary. This will be considered further in Chapter VI.

CHAPTER IV

Filter-feeding of carps on planktonic algae

4.1. Introduction

The Indian and Chinese carp rohu, *Labeo rohita*; catla, *Catla catla* and silver carp, *Hypophthalmichthys molitrix* are principally filter feeding planktivores (Khan and Siddiqui, 1973; Jhingran and Pullin, 1985; Pillay, 1990). There have been increasing movements of Chinese and Indian major carps for aquaculture purposes and in many countries, particularly in Asia, the two groups of carp are stocked in combination in fish ponds and, because of escapes, occur together in the wild.

As stated in the Introduction, filter-feeding Chinese carps appear to out-perform the Indian major carps in terms of growth and survival, when stocked together in polyculture. Much work has been done on the food and feeding habits of carps (*e.g.* Khan and Siddiqui, 1973; Jafri and Mustafa, 1977; Spataru and Gophen, 1985; Dewan *et al.*, 1991). Although all have been qualitative in nature, they have clearly demonstrated significant dietary overlap among many species, particularly between silver carp and rohu, bighead carp and catla. Quantitative studies of algal ingestion are needed in order to establish the basis of competition and these can only be carried out

in the laboratory.

There have been numerous laboratory based quantitative studies of filter feeding, although mainly on zooplankton (McMahon and Rigler, 1963; Frost, 1972; Crowley, 1973; Frost, 1975; Reeve and Walter, 1977; Conover and Mayzaud, 1984) and anuran larvae (tadpoles) (Seale and Wassersug, 1979; Seale and Beckvar, 1980; Seale *et al.*, 1982). A little work on quantitative aspects of filter feeding has been carried out on some fishes (Moriarty and Moriarty, 1973; Durbin and Durbin, 1975; Drenner *et al.*, 1982; Friedland, 1984; McDonald, 1985; Drenner *et al.*, 1987; Northcott *et al.*, 1991) but none has been done on carps.

The results of quantitative filter-feeding studies have been used to create functional response models. There are several curves (rectilinear, Ivlev, Michaelis-Menten) used for simplified representation of their experimental data.

The basis for mathematical models of filter-feeding in fishes is largely derived from zooplankton studies by Frost (1972). The ingestion rate equation was further developed and tested by Conover and Mayzaud (1984), Peters (1984), and Marin *et al.* (1986) and most recently has been used by Northcott *et al.* (1991) for work with the tilapia *O. niloticus*. The advantage of this form of equation is that it is based on actual net changes in algal concentrations in the experimental feeding chamber.

There are several ways to express the ingestion-concentration curve. Frost (1972) used cell number whereas Crowley (1973) used dry weight of food particles in the ingestion-concentration curve. Lehman (1976) proposed an energy optimization theory and according to his hypothesis, ingestion is related to the energy content of the suspended food particles. Seale and Beckvar (1980) state that tadpoles regulate their food intake on the basis of volume or biomass ingested. Northcott *et al.* (1991) also reported that ingestion by the tilapia *O. niloticus* is regulated by bio-volume of the food particles.

Filter-feeding fishes feed on blue-green algae (Cyanobacteria) along with other algae and zooplankton (Moriarty, 1973; Kajak, *et al.*, 1977; Spataru and Zorn , 1978; Bowen, 1982). Silver carp are capable of ingesting small blue-green algal species such as *Microcystis aeruginosa* (Kajak, *et al.*, 1975). Many of the blue-green algae which proliferate in eutrophic fresh- and brackish waters, however, produce powerful hepato- and neurotoxins which can cause mortalities when ingested by animals (Skulberg, *et al.*, 1984; Codd, *et al.*, 1989). At present it is not known whether phytoplankton-feeding fishes are capable of detecting or ingesting toxic cyanobacteria and if so , how they deal with ingested toxins.

It was thus decided to carry out a quantitative filter-feeding study on the fry of *L. rohita*, *C. catla*, and *H. molitrix*. The ingestion rates at various concentrations of *Pediastrum boryanum* and *Microcystis aeruginosa* were measured in an attempt to produce functional response curves. In a separate series of trials an investigation was

carried out to determine whether phytoplankton-feeding silver carp could detect and ingest toxic Cyanobacteria.

4.2 Materials and Methods

4.2.1 Introduction

Two sets of experiments were carried out. The first set was designed to evaluate the effects of particle size and density on ingestion rates of the three species of carps. A second set of experiments was designed to investigate whether silver carp are able to detect and ingest toxic *M. aeruginosa*.

4.2.1 Algae

The species used in this experiment were the green alga *Pediastrum boryanum*, and toxic and non toxic strains of the Cyanobacterium *Microcystis aeruginosa*. These algae occur naturally in tropical freshwater environments and are typical of those prevailing in carp culture systems. Culture methods and maintenance of algae are as described in Chapter- II. The toxic strain of *M. aeruginosa* was obtained from the Department of Biology, University of Dundee.

4.2.2 Fish

All three fish species, rohu (*L. rohita*), catla (*C. catla*) and silver carp (*H. molitrix*) were used in the quantitative investigation of algal ingestion. Silver carp only were used to study whether fish can detect or ingest toxic strains of *M. aeroginosa*. Fish were reared in the Tropical Aquarium, Institute of Aquaculture (see Chapter III). Two size classes of fish, 1.4 and 7.7 g of rohu, 1.7 and 8.6 g of catla, and 1.1 and 7.1 g of silver carp were used for the quantitative feeding trial. Silver carp of 5 g size were used in the investigation of toxic algal ingestion.

4.2.3 Experimental System

The experimental system consisted of 16 tanks of 30 l capacity incorporated into a recirculation system and static water (*i.e.* no water exchange takes place) system where the filter-feeding trials were performed (Fig.4.1). Each tank in the recirculation system contained two static 2 l feeding chambers. The temperature of the feeding chambers was maintained at 26 ± 1°C. Air was supplied via separate air supply tubes to each feeding chamber and the supply regulated by plastic screw clamps. Each feeding chamber was fitted with a perspex lid with a small hole in the centre to facilitate air exchange. An overhead fluorescent tube was fitted to ensure an adequate light (120 Lux) supply. Each tank was surrounded by black polythene sheet to protect

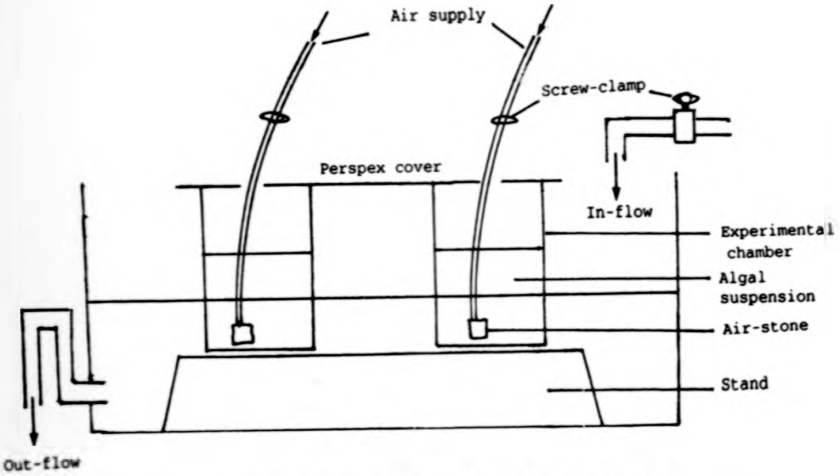


Fig. 4.1 Diagram of the system used in the filter feeding trials.

the fish from undue external visual disturbance.

4.2.4 Experimental Protocol

Ingestion trials:

In all trials filtered tap water was used. Stocking densities were 6.73 ± 1.74 g fish, l^{-1} except for the trials involving toxic algae. The trials were single end-point experiments, *i.e.* the algal density in the feeding chambers was recorded at time zero and then again at the end of the feeding period. All feeding trials were of 4 h duration. Five replicates for small fish (1.1-1.7 g), three replicates for large fish (7.1 - 8.6 g) and three controls were used for each algal concentration (5 concentrations, 9 - 193 x 10^6 μm^3, ml^{-1}). All trials were started at 10.00 hours.

Selected fish were moved to the static system and starved for 12- 24 hours. Two hours before the filter-feeding trial fish were transferred to their respective feeding chambers. Two water samples of 500 µm were taken from each feeding chamber, mixed together with 20 ml ISOTON II and analyzed by Coulter Multisizer in order to determine the background particle count (i.e. sample blank).

Immediately prior to each trial the algal density of the harvest culture was estimated by Coulter Multisizer. In order to obtain the desired algal density in the feeding chamber the quantities of culture that should be added were determined and diluted

with filtered tap water prior to adding to the feeding chamber. Actual initial concentrations in the experimental chambers were determined from samples taken 5 min after addition of the algae in order to allow for thorough mixing. Two samples (initial), each consisting of two 500 µl samples were taken from each chamber and added to 20 ml ISOTON II for counting and another one 2 ml sample was taken randomly from each treatment for size distribution pattern of algal colonies. The fish were allowed to feed on the algae for 4 h in all trials. Final samples were taken at the end of this period in the same way as the initial samples. On all occasions one fish from each replicate was sacrificed and the anterior portion of the gut dissected out in order to verify whether the fish had been filter-feeding. The volume of water and the total weight of the fishes in each feeding chamber were recorded.

Trials involving toxic algae:

Individual silver carp (5 g) were used in the same experimental system. Toxic and non-toxic strains of *M. aeruginosa* were used as separate treatments. Seven or eight replicates for each treatment and three replicates for control were used in the experiments. Sampling was done as for the previous trials (see above).

Individual fish of the same size were stocked in small static flow tanks as described above, except that no black polythene was used around the tank sides in order to facilitate observation of opercular beat rates. Three treatments were used (control [fish,

no Cyanobacteria], fish + toxic Cyanobacteria and fish + non-toxic Cyanobacteria) and three replicates for each treatment established. Fish were starved for 12 h prior to transfer to the experimental system, they were allowed 2 h to acclimate. Cyanobacteria were then introduced to all tanks other than the controls. Initial Cyanobacterial concentrations were approximately 10^6 cell, ml^{-1}. Observations were made immediately after the introduction of Cyanobacteria (0 h) and after 4 h. Observations were made for period of 1 min using a stopwatch, three fish being observed for each treatment at each time interval, and fish being selected at random. Care was taken not to disturb fish.

Samples (500 ml) of water from experimental tanks and from controls were passed through C_{18} Sep-Pak cartridges (Water Associates) to concentrate any microcystin-LR which may have been present. Cartridge eluates were analysed by reverse-phase HPLC (Water Associates) using purified microcystin-LR as standard at the Department of Biological Sciences, University of Dundee.

4.2.5 Sample Analysis

All samples were analyzed in fresh condition immediately after sampling. The Coulter Multisizer was used to determine algal density. At least three counts were made for each sample. All counts were corrected by deducting the blank count value. For the determination of algal cell size, a high powered (x 400) light microscope with eyepiece graticule was used (see Chapter II)

4.2.6 Determination of Ingestion rates

Ingestion rates were calculated in terms of algal mean bio-volume rather than the cell or colony number so that changes in cell or colony size during the experiment could be taken into account.

Ingestion rates were computed using the equation proposed by Conover & Mayzaud (1984) and Peters (1984) :

$$I = V/W((C_oB_o - C_tB_t)/\Delta t)$$

where I = ingestion rate ($\mu m^3 g^{-1} h^{-1}$); C_o = initial algal concentration in experimental chamber (colonies or cells ml^{-1}); C_t = final algal concentration in experimental chamber (colonies or cells ml^{-1}); B_o = mean algal colony or cell biovolume at start of feeding period (μm^3); B_t= mean algal colony or cell biovolume at end of feeding period (μm^3); Δt = experimental duration (h); V = volume of algal suspension (ml); and W = wet weight of fish in experimental container (g).

No correction was made for algal reproduction due to the short duration of the experiments (4 h).

Mean algal bio-volume during the feeding period for each replicate was calculated

using the equation from Northcott *et al.* (1991):

$$B_e = (C_t B_t - C_o B_o)/\ln(C_t B_t/C_o B_o)$$

Where B_e = mean algal biovolume concentration ($\mu m^3 ml^{-1}$).

The dependence of ingestion rate on algal concentration was described by the sigmoid model used by Kiørboe *et al* (1985) for zooplankton ingestion data:

$$I = I_{max}\, e^{-b/C}$$

where I_{max} = maximum ingestion rate; C = algal concentration; b = a constant. The model was fitted to experimental data by linear regression after logarithmic transformation.

4.3 Results

4.3.1 Algal ingestion trials

Five different treatments, each consisting of a different initial concentration of algae (9 -193 x 10^6 μm^3. ml^{-1}), were used for each species of fish. For all feeding trial treatments involving silver carp and rohu a Student's *t*-test on initial versus final algal

biovolume concentrations showed a significant decrease (Table-4.1) indicating that grazing on both *M. aeruginosa* and *P. boryanum* had taken place in the experimental chambers. The ingestion of algae was verified by the presence of algae in the stomachs at end of the feeding period. In the trials involving catla and *M. aeruginosa* no significant decrease ($P > 0.05$) at the end of the feeding trials was apparent, indicating that no grazing had taken place.

Throughout all feeding trials involving *M. aeruginosa*, colony bio-volume was constant and there was no evidence of size selection by the fish. Although both *M. aeruginosa* and *P. boryanum* were harvested at the end of the log phase of the culture, it was found that there was some size variation among the *P. boryanum* colonies, although the size distribution pattern was similar among replicates. From the data in Tables 4.2, 4.3 and 4.4 it is apparent that there were significant differences in *P. boryanum* mean colony diameter among certain treatments. The results also indicate significant decreases in mean colony size in some treatments during the feeding trials. For each species of fish and size group, ingestion rate I was plotted against the corresponding algal biovolume concentration B_c for each species of algae (Fig. 4.2 - 4.11). Ingestion rate versus algal food concentration curves were produced on the basis of the equation $I = I_{max} e^{-kC}$. The ingestion rate of rohu (7.7 g) feeding on *M. aeruginosa* and catla (1.7 g) feeding on *P. boryanum* showed highest correlation coefficients ($r^2 = 0.97$ and 0.98 respectively).

Table 4.1 Student's t- test values for initial versus final algal concentration of different experimental treatments of (a) *L. rohita*, (b) *C. catla* and (c) *H. molitrix*

(a)

1.4 g fish		7.7 g fish		
Algal concentration (n) (biovolume x 10^6) ± SD 1 = initial, 2 = final	t	Algal concentration (n) (biovolume x 10^6) ± SD 1 = initial, 2 = final	t	Algal species
1. 8.99 ± 0.08 (5) 2. 8.71 ± 0.03	1.72	1. 9.81 ± 0.01 (3) 2. 8.97 ± 0.08	4.17*	*M. aeruginosa*
1. 46.61 ± 0.12 (5) 2. 45.64 ± 0.46	4.01*	1. 46.52 ± 0.21 (3) 2. 45.12 ± 0.97	2.33	"
1. 95.99 ± 0.22 (5) 2. 92.38 ± 0.50	11.63***	1. 97.13 ± 0.14 (3) 2. 95.08 ± 1.12	3.07*	"
1. 137.45 ± 0.22 (5) 2. 133.33 ± 0.58	10.80***	1. 137.49 ± 0.08 (3) 2. 134.34 ± 0.57	9.19***	"
1. 193.05 ± 0.13 (5) 2. 187.85 ± 2.03	8.57*	1. 192.98 ± 0.05 (3) 2. 189.15 ± 1.67	3.96*	"
1. 9.19 ± 0.04 (6) 2. 7.16 ± 0.30	6.49**	1. 9.13 ± 0.04 (3) 2. 7.62 ± 0.34	4.68*	*P. boryanum*
1. 45.77 ± 0.30 (6) 2. 41.31 ±0.54	4.44 *	1. 46.37 ± 0.10 (3) 2. 41.65 ± 0.40	7.20**	"
1. 97.52 ± 0.36 (6) 2. 88.14 ±0.99	6.11**	1. 97.79 ± 0.62 (3) 2. 89.41 ± 0.46	3.48*	"
1. 137.72 ± 0.24 (6) 2. 127.07 ± 0.98	4.31*	1. 137.93 ± 0.15 (3) 2. 126.71 ± 1.12	3.84*	"
1. 195.07 ± 0.33 (6) 2. 179.52 ± 1.86	3.78*	1. 194.18 ± 0.37 (3) 2. 178.84 ± 2.48	2.11	"

* = 0.05 > P > 0.01; ** = 0.01 > P > 0.001; *** = P <0.001

(b) *C. catla.*

1.7 g fish		8.6 g fish		
Algal concentration (n) (biovolume x 10^6) ± SD 1 = initial, 2 = final	t	Algal concentration (n) (biovolume x 10^6) ± SD 1 = initial, 2 = final	t	Algal species
1. 9.02 ± 0.05 (4) 2. 8.96 ± 0.03	2.08	1. 9.06 ± 0.01 (3) 2. 9.03 ± 0.05	1.11	*M. aeruginosa*
1. 46.59 ± 0.13 (4) 2. 46.59 ± 0.09	0.23	1. 46.61 ± 0.10 (3) 2. 46.59 ± 0.13	0.22	"
1. 96.52 ± 0.76 (4) 2. 96.36 ± 0.61	0.31	1. 96.14 ± 0.13 (3) 2. 96.12 ± 0.11	0.40	"
1. 136.93 ± 0.98 (4) 2. 136.86 ± 0.98	0.04	1. 137.22 ± 0.20 (3) 2. 137.17 ± 0.11	0.62	"
1. 193.00 ± 0.11 (4) 2. 192.96 ± 0.13	0.43	1. 192.85 ± 0.03 (3) 2. 192.84 ± 0.07	0.06	"
1. 9.10 ± 0.06 (4) 2. 7.67 ± 0.22	7.56**	1. 9.13 ± 0.04 (3) 2. 7.80 ± 0.27	7.24**	*P. boryanum*
1. 45.98 ± 0.24 (4) 2. 41.52 ± 0.75	3.59*	1. 46.26 ± 0.08 (3) 2. 41.42 ± 0.52	9.67***	"
1. 97.47 ± 0.65 (4) 2. 88.80 ± 1.36	2.80*	1. 97.07 ± 0.13 (3) 2. 88.41 ± 0.50	12.79***	"
1. 138.70 ± 0.50 (4) 2. 127.44 ± 1.42	2.97*	1. 137.95 ± 0.60 (3) 2. 126.92 ± 1.14	5.60**	"
1. 194.47 ± 0.22 (4) 2. 179.51 ± 1.73	3.06*	1. 194.76 ± 0.08 (3) 2. 179.81 ± 2.63	4.18*	"

* = $0.05 > P > 0.01$; ** = $0.01 > p > 0.001$; *** $p < 0.001$

(c) *H. molitrix*.

1.1 g fish		7.1 g fish		
Algal concentration (n) (biovolume x 10^4) ± SD 1 = initial, 2 = final	t	Algal concentration (n) (biovolume x 10^4) ± SD 1 = initial, 2 = final	t	Algal species
1. 9.03 ± 0.03 (5) 2. 8.50 ± 0.32	3.56*	1. 9.09 ± 0.02 (4) 2. 8.63 ± 0.22	4.11*	*M. aeruginosa*
1. 46.52 ± 0.13 (5) 2. 44.76 ± 1.27	2.99*	1. 46.59 ± 0.13 (4) 2. 44.71 ± 1.31	2.79*	"
1. 96.80 ± 0.21 (5) 2. 93.13 ± 1.58	3.65*	1. 96.95 ± 0.12 (4) 2. 92.82 ± 0.98	8.09**	"
1. 137.56 ± 0.16 (5) 2. 132.14 ± 1.00 (5)	9.73**	1. 137.46 ± 0.15 (4) 2. 132.08 ± 1.10	9.42***	"
1. 192.93 ± 0.03 (5) 2. 187.52 ± 2.31	4.30**	1. 192.89 ± 0.01 (4) 2. 186.62 ± 1.17	10.64***	"
1. 9.08 ± 0.04 (4) 2. 7.16 ± 0.54	6.06**	1. 9.13 ± 0.06 (4) 2. 6.78 ± 0.51	3.81*	*P. boryanum*
1. 46.28 ± 0.06 (4) 2. 41.31 ± 0.54	9.06**	1. 46.18 ± 0.03 (4) 2. 41.43 ± 0.72	5.11**	"
1. 96.94 ± 0.18 (4) 2. 88.14 ± 0.99	6.09**	1. 97.18 ± 0.14 (4) 2. 88.16 ± 1.00	5.62**	"
1. 137.90 ± 0.04 (4) 2. 125.79 ± 1.14	5.96**	1. 137.94 ± 0.03 (4) 2. 126.08 ± 0.88	7.26**	"
1. 195.01 ± 0.30 (4) 2. 178.48 ± 1.57	6.16**	1. 194.56 ± 0.08 (4) 2. 178.00 ± 1.67	5.16**	"

* = $0.05 > P > 0.01$; ** = $0.01 > P > 0.001$; *** = $P < 0.001$.

Table 4.2 Results of Student's *t*-test carried out on *P. boryanum* colony diameter before and after grazing. (a) 1.4 g *L. rohita*, (b) 7.7 g *L. rohita*.

Treatment mean biovolume Bc ($\mu m^3 ml^{-1}$)	Mean colony diameter ± SD (μm) 1. Start (n) 2. End (n)	t	p
(a)			
9.15×10^6	1. 63.76 ± 9.45 (110) 2. 61.64 ± 8.36 (110)	1.42	>0.1
4.62×10^7	1. 63.08 ± 10.47 (110) 2. 61.65 ± 9.03 (110)	1.01	>0.1
9.62×10^7	1. 62.38 ± 9.35 (115) 2. 61.02 ± 8.14 (112)	1.02	>0.1
13.67×10^7	1. 64.18 ± 8.68 (115) 2. 61.78 ± 7.91 (115)	2.01	<0.05
19.48×10^7	1. 61.77 ± 10.69 (110) 2. 58.52 ± 8.62 (110)	2.47	<0.02
(b)			
9.05×10^6	1. 61.27 ± 9.26 (120) 2. 59.38 ± 8.56 (120)	1.14	>0.1
4.61×10^7	1. 53.08 ± 10.39 (110)* 2. 51.14 ± 9.63 (110)	1.24	>0.1
9.67×10^7	1. 64.02 ± 9.59 (140) 2. 61.76 ± 8.34 (140)	2.01	<0.05
13.77×10^7	1. 52.43 ± 10.03 (110)* 2. 51.01 ± 9.27 (110)	1.01	>0.1
19.41×10^7	1. 64.06 ± 9.46 (115) 2. 61.41 ± 8.37 (115)	2.08	<0.05

* Colonoy diameter was low, however bio-volume density was addjusted by increasing the colonoy number.

Table 4.3 Results of Student's *t*-test carried out on *P. boryanum* colony diameter before and after grazing. (a) 1.7 g *C. catla*, (b) 8.6 g *C. catla*

Treatment mean biovolume Bc ($\mu m^3 ml^{-1}$)	Mean colony diameter (μm) ± SD 1. Start (n) 2. End (n)	t	P
(a)			
9.14×10^6	1. 63.98 ± 9.56 (124) 2. 62.04 ± 9.23 (124)	1.31	>0.05
4.62×10^7	1. 64.03 ± 9.21 (110) 2. 62.87 ± 8.63 (110)	1.69	>0.05
9.93×10^7	1. 63.02 ± 9.55 (120) 2. 61.14 ± 9.02 (120)	1.38	>0.05
13.96×10^7	1. 63.41 ± 9.33 (121) 2. 60.39 ± 8.03 (121)	2.06	<0.05
19.49×10^7	1. 64.19 ± 10.9 (115) 2. 60.76 ± 9.28 (115)	2.49	<0.05
(b)			
9.06×10^6	1. 63.92 ± 9.74 (146) 2. 62.52 ± 9.19 (146)	1.26	>0.1
9.73×10^7	1. 56.99 ± 9.7 (140) 2. 54.96 ± 9.13 (140)	1.8	>0.05
4.64×10^7	1. 64.2 ± 10.11 (110) 2. 61.72 ± 9.13 (110)	2.67	<0.05
13.79×10^7	1. 62.27 ± 10.11 (112) 2. 59.73 ± 10.02 (112)	1.87	>0.05
19.47×10^7	1. 62.24 ± 9.65 (115) 2. 61.17 ± 8.56 (115)	1.06	>0.1

Table 4.4 Results of Student's t-test carried out on *P. borynum* colony diameter before and after grazing. (a) 1.1 g *H. molitrix*, (b) 7.1 g *H. molitrix*

Treatment mean biovolume Bc ($\mu m^3 ml^{-1}$)	Mean colony diameter ± SD (μm) 1. Start (n) 2. End (n)	t	p
(a)			
9.18×10^6	1. 63.86 ± 9.41 (110) 2. 61.47 ± 8.59 (110)	1.43	>0.1
4.61×10^7	1. 61.28 ± 8.71 (115) 2. 59.62 ± 7.53 (115)	1.03	>0.1
9.75×10^7	1. 62.53 ± 9.26 (120) 2. 60.81 ± 8.34 (120)	1.17	>0.1
13.77×10^7	1. 64.03 ± 8.64 (110) 2. 62.01 ± 8.01 (110)	1.43	>0.1
19.46×10^7	1. 62.52 ± 10.26 (115) 2. 60.17 ± 9.06 (115)	1.12	>0.1
(b)			
9.02×10^6	1. 62.36 ± 8.34 (110) 2. 61.03 ± 7.03 (110)	1.01	>0.1
4.61×10^7	1. 57.26 ± 9.48 (110) 2. 55.81 ± 8.65 (110)	1.42	>0.1
9.74×10^7	1. 63.21 ± 9.06 (110) 2. 61.08 ± 8.45 (110)	1.06	>0.1
13.79×10^7	1. 61.76 ± 8.64 (100) 2. 59.42 ± 8.03 (100)	1.02	>0.1
19.44×10^7	1. 63.04 ± 9.26 (100) 2. 59.23 ± 8.04 (100)	2.03	<0.05

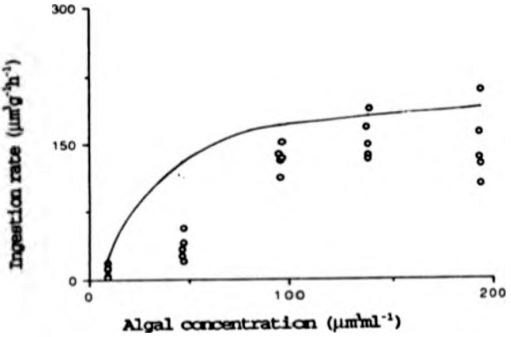


Fig. 4.2 Ingestion rate (I) as a function of food concentration (C) for *L. rohita* (1.4 g) feeding on *M. aeruginosa*. Fitted curve is $I = 2.1E+08.e^{-2.1E+07/C}$, ($r^2$ = 0.88).

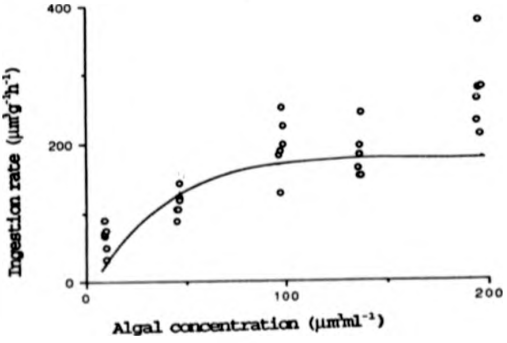


Fig. 4.3 Ingestion rate (I) as a function of food concentration (C) for *L. rohita* (1.4 g) feeding on *P. boryanum*. Fitted curve is $I = 2.1E+08.e^{-2.1E+07C}$, ($r^2$ = 0.88).

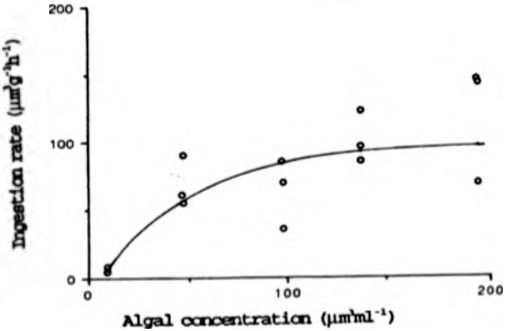


Fig. 4.4 Ingestion rate (I) as a function of food concentration (C) for *L. rohita* (7.7 g) feeding on *M. aeruginosa*. Fitted curve is $I = 1.1E+08.e^{-2.48E+07/C}$, ($r^2$ = 0.97).

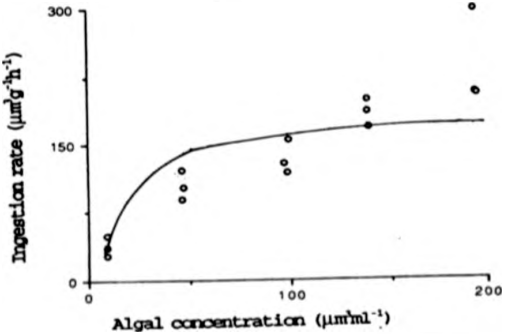


Fig. 4.5 Ingestion rate (I) as a function of food concentration (C) for *L. rohita* (7.7 g) feeding on *P. boryanum*. Fitted curve is $I = 1.9E+08.e^{(-1.5E+07/C)}$, ($r^2$ = 0.90).

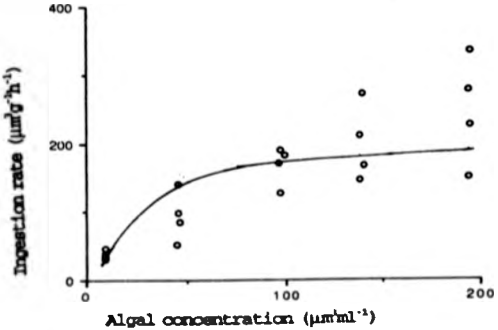


Fig. 4.6 Ingestion rate (I) as a function of food concentration (C) for *C. catla* (1.7 g) feeding on *P. boryanum*. Fitted curve is $I = 1.8E+08.e^{-4.7E+01C}$, ($r^2$ = 0.98).

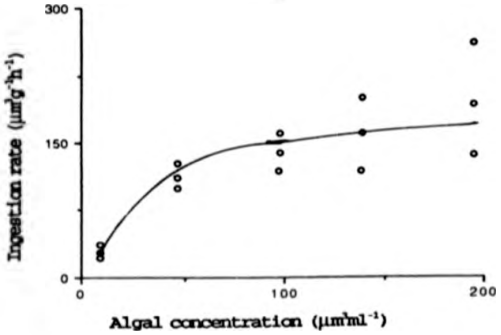


Fig. 4.7 Ingestion rate (I) as a function of food concentration (C) for *C. catla* (8.6 g) feeding on *P. boryanum*. Fitted curve is $I = 2.0E+08.e^{-1.6E+07/C}$, ($r^2$ = 0.88).

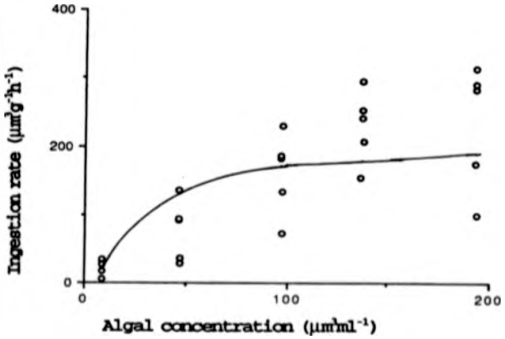


Fig. 4.8 Ingestion rate (I) as a function of food concentration (C) for *H. molitrix* (1.1 g) feeding on *M. aeruginosa*. Fitted curve is $I = 2.1E+08.e^{-2.1E+07/C}$, ($r^2$ = 0.88).

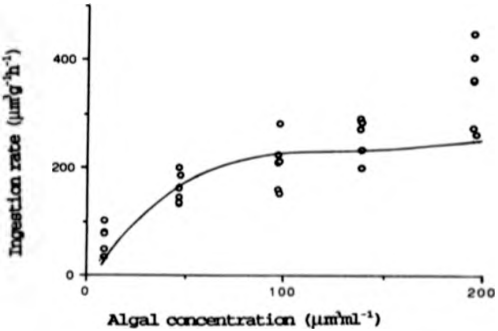


Fig. 4.9 Ingestion rate (I) as a function food concentration (C) for *H. molitrix* (1.1 g) feeding on *P. boryanum*. Fitted curve is $I = 2.7E+08.e^{-2.1E+07/C}$, ($r^2$ = 0.89).

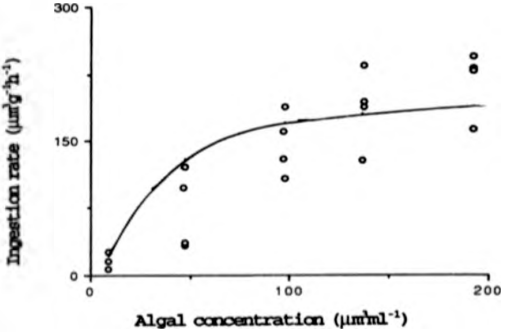


Fig. 4.10 Ingestion rate (I) as a function of food concentration (C) for *H. molitrix* (7.1 g) feeding on *M. aeruginosa*. Fitted curve is $I = 2.1E+08.e^{-2.11E+07/C}$, ($r^2$ = 0.88).

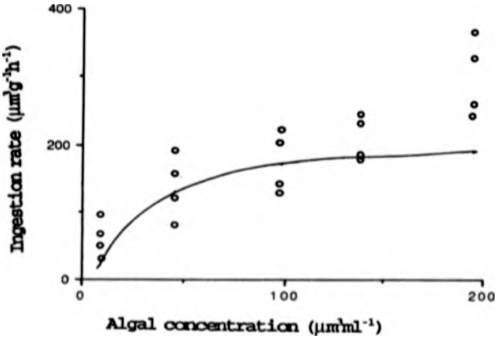


Fig. 4.11 Ingestion rate (I) as a function of food concentration (C) for *H. molitrix* (7.1 g) feeding on *P. boryanum*. Fitted curve is $I = 2.1E{+}08.e^{-2.1E+07/C}$, ($r^2$ = 0.88).

4.3.2 Toxic algal ingestion trials

Ingestion of toxic and non-toxic Cyanobacteria:

In the trial in which silver carp were exposed to both toxic and non-toxic strains of the planktonic Cyanobacterium *M. aeruginosa* the results clearly show that grazing on toxic *M. aeruginosa* was significantly depressed in comparison to grazing rates on the non toxic strain (ANOVA, d.f. 1, 25, $F = 45.6$, $p < 0.05$) (Fig.4.12). Indeed, there was no significant difference (Tukey MR Test, $P < 0.05$) in Cyanobacterial cell numbers at the end of the trial between the toxic treatment and the control consisting of toxic *M. aeruginosa* with no fish. Gut content analysis showed that very few cells ($<10^5$) of the toxic stain of *M. aeruginosa* had been ingested, compared with fish exposed to the non-toxic strain ($>10^7$).

Behaviourial response:

The results from timed measurements of opercular beat rates at 0 h are shown in Fig. 4.13. The behaviours of fish held in toxic Cyanobacteria did not differ significantly from the controls in which fish were held in media without *M. aeruginosa* whereas those of fish held in non-toxic Cyanobacteria were significantly elevated (ANOVA d.f. 2, 26, $F = 83.3$, $P < 0.05$; Tukey MR Test, $P < 0.05$). The same statistically significant results were observed at 4 h.

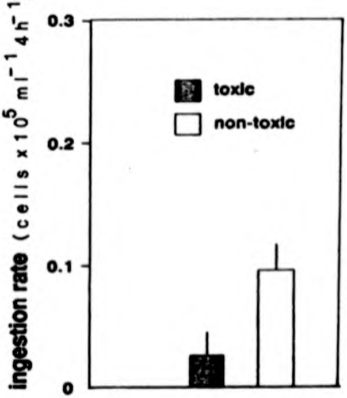


Fig. 4.12 Ingestion rates (cells x $10^5 ml^{-1} 4h^{-1}$) of silver carp (*H. molitrix*) exposed to toxic and non-toxic *M. aeruginosa*.

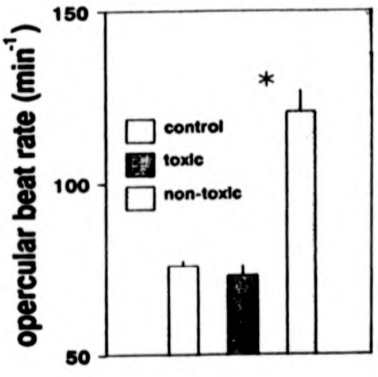


Fig. 4.13 The influence of toxic and non-toxic strains of *M. aeruginosa* on opercular beat rate in *H. molitrix*. Bars denote mean (+ 1 s.d.) opercular beat rates.

Water analysis:

Analysis of samples of water from the experimental chambers and from the controls by HPLC confirmed that no detectable levels of microcystin (< 250 ng l^{-1}) were present in the water.

4.4 Discussion

Functional response models have been used to describe and explain ingestion versus food concentration relationships in many different types of filter feeders, including fish (Durbin and Durbin, 1975; Drenner *et al.*, 1987; Northcott *et al.*, 1991), anuran larvae (Seale and Wassersug, 1979; Seale and Beckvar 1980) and zooplankton (Frost, 1972; Crowley, 1973; Robertson and Frost, 1977; Kiorboe *et al.* 1985). Whilst there remains some disagreement about the exact nature of the relationship - rectilinear, as suggested by Holling (1959, 1965, 1966) or curvilinear as proposed by Ivlev (1961) for fish - Mullin *et al.* (1975) have found that for zooplankton at least the ingestion versus concentration relationship can be described by both curvilinear and rectilinear models; in terms of fit, they are statistically indistinguishable. Indeed, they also showed that a Michaelis-Menten type curve can be justifiably fitted to available data. Seale *et al.* (1982) also suggested that the fundamental regulatory mechanisms such as satiation or gut fullness are similar between invertebrate and tadpole suspension feeders. The results of this study appear to confirm this universality in fundamental regulatory mechanisms among filter feeders.

Whilst the model applied here does not seem to fit some of the data sets particularly well, overall it fits the data adequately and other than examination of residuals after model fitting there are no appropriate methods for testing goodness of fit among curvilinear data (Sokal and Rohlf, 1973). The ingestion versus food concentration curves of small rohu (1.4 g) and small silver carp (1.1 g) feeding on *M. aeruginosa* (Fig. 4.2 and Fig. 4.8) and small catla feeding on *P. boryanum* (Fig. 4.6) seem to reach an asymptotic level which is typical for filter feeders and is compatible with a curvilinear model where filtering surfaces become fully saturated at very high food concentrations. Ingestion versus food concentration curves for small rohu and silver carp feeding on *P. boryanum* (Fig. 4.3 and Fig. 4.9), however, appear not to reach an asymptotic level, suggesting that the concentration of *P. boryanum* at which saturation ingestion occurs had not been reached. Similar relationships were also observed for large rohu and silver carp feeding on both *M. aeruginosa* and *P. boryanum* and large catla feeding on *P. boryanum*. Durbin and Durbin (1975) found that their Atlantic menhaden data fitted the linear phase of the feeding curve. Ishii *et al.* (1985) found that the ingestion rate of *Euphausia superba* Dana increased linearly with increasing particle concentration over the range of food concentrations used. Northcott *et al* (1991) also found a linear relationship for the ingestion data of large *O. niloticus* feeding on both *Microcystis* and *Anabaena*.

Ingestion rates for rohu, catla and silver carp feeding on *P. boryanum* were higher than for those feeding on *M. aeruginosa* (Table 4.5), suggesting that the fish were more

efficient at feeding on larger algae. Previous studies found that ingestion rate increased with particle size for *Sarotherodon galilaeus* (Drenner *et al.*, 1987) and *O. niloticus* (Northcott *et al.*, 1991).

The ingestion rates of silver carp feeding on both *M. aeruginosa* and *P. boryanum* were higher than the ingestion rates of rohu and catla feeding on the same alga at the same concentrations (Table 4.5). This may be because the filtering apparatus of silver carp is more efficient than that of rohu and catla. Seale and Beckvar (1980) reported that the tadpole of one anuran species, *Xenopus laevis*, had a maximum ingestion rate significantly higher than the others. *Xenopus* tadpoles have an extensive area of mucus secretory ridges in the buccal region (Seale and Wassersug, 1979), suggesting a more efficient mucus entrapment process. Results from Chapter III show that the inter-gill raker spacings of silver carp are less than those of rohu and catla. These points are discussed further in Chapter VI.

In all trials rohu and silver carp were observed to ingest both *P. boryanum* and *M. aeruginosa*. Catla however, were unable to ingest *M. aeruginosa*. This may be because gill raker spacings in catla are greater than those of corresponding sizes of rohu and silver carp and are thus unable to retain *M. aeruginosa*. Alternatively, catla may reject *M. aeruginosa* as food because it may not be digestible. Catla show the ability to selectively feed on zooplankton in the natural environment (Jafri and Mustafa, 1977; Jhingran and Pullin, 1985; Pillay, 1990). Ingestion of *M. aeruginosa* by rohu and silver

Table 4.5 Ingestion rate for different species feeding on different algae at different concentrations, (a) *L. rohita*, (b) *C. catla*, (c) *H. molitrix*.

(a)

Algal species	Fish size (g)	Algal concentration ($\mu m^3 ml^{-1}$) $x10^6$ ± SE	Ingestion rate ($\mu m^3 g^{-1} h^{-1}$) $x10^6$ ± SE
M. aeruginosa	1.4	8.99 ± 0.08	10.33 ± 2.88
P. boryanum	1.4	9.19 ± 0.04	63.93 ± 8.12
M. aeruginosa	7.7	9.18 ± 0.01	6.61 ± 1.27
P. boryanum	7.7	9.13 ± 0.04	37.82 ± 6.10
M. aeruginosa	1.4	46.61 ± 0.12	35.43 ± 6.38
P. boryanum	1.4	45.77 ± 0.30	97.20 ± 8.76
M. aeruginosa	7.7	46.52 ± 0.21	44.36 ± 13.81
P. boryanum	7.7	46.37 ± 0.10	89.09 ± 7.59
M. aeruginosa	1.4	95.99 ± 0.22	133.35 ± 6.40
P. boryanum	1.4	97.52 ± 0.36	174.47 ± 16.39
M. aeruginosa	7.7	97.13 ± 0.14	63.69 ± 14.65
P. boryanum	7.7	97.79 ± 0.62	133.60 ± 10.98
M. aeruginosa	1.4	137.45 ± 0.22	154.74 ± 10.31
P. boryanum	1.4	137.72 ± 0.24	181.93 ± 14.25
M. aeruginosa	7.7	137.49 ± 0.08	100.95 ± 10.96
P. boryanum	7.7	137.93 ± 0.15	183.47 ± 8.65
M. aeruginosa	1.4	193.05 ± 0.13	159.40 ± 18.04
P. boryanum	1.4	195.07 ± 0.33	273.16 ± 23.51
M. aeruginosa	7.7	192.98 ± 0.05	199.14 ± 25.05
P. boryanum	7.7	194.18 ± 0.37	235.68 ± 31.01

(b)

Algal species	Fish size (g)	Algal concentration ($\mu m^3 ml^{-1}$) $x10^6$ ± ES	Ingestion rate ($\mu m^3 g^{-1} h^{-1}$) $x10^6$ ± SE
P. boryanum	1.7	9.10 ± 0.06	37.76 ± 3.34
P. boryanum	8.6	9.13 ± 0.04	28.30 ± 4.33
P. boryanum	1.7	45.98 ± 024	93.55 ± 18.58
P. boryanum	8.6	46.26 ± 0.08	84.22 ± 8.57
P. boryanum	1.7	97.47 ± 0.65	167.47 ± 14.36
P. boryanum	8.6	97.09 ± 0.13	139.34 ± 12.29
P. boryanum	1.7	138.70 ± 0.50	198.61 ± 28.15
P. boryanum	8.6	137.95 ± 0.06	159.44 ± 23.81
P. boryanum	1.7	194.47 ± 0.20	246.99 ± 39.45
P. boryanum	8.6	194.76 ± 0.03	195.81 ± 36.16

(c)

Algal species	Fish size (g)	Algal concentration (µm3ml-1)x106 ± SE	Ingestion rate (µm3g-1h-1)x106 ± SE
M. aeruginosa	1.1	9.03 ± 0.03	22.87 ± 5.18
P. boryanum	1.1	9.08 ± 0.04	69.74 ± 9.98
M. aeruginosa	7.1	9.09 ± 0.02	16.25 ± 3.85
P. boryanum	7.1	9.13 ± 0.06	60.94 ± 13.92
M. aeruginosa	1.1	46.52 ± 0.13	76.83 ± 19.78
P. boryanum	1.1	46.28 ± 0.06	133.58 ± 10.80
M. aeruginosa	7.1	46.59 ± 0.13	67.78 ± 20.59
P. boryanum	7.1	46.18 ± 0.03	99.34 ± 12.80
M. aeruginosa	1.1	96.80 ± 0.21	141.12 ± 20.94
P. boryanum	1.1	96.94 ± 0.18	206.50 ± 19.36
M. aeruginosa	7.1	96.95 ± 0.12	146.07 ± 17.55
P. boryanum	7.1	97.18 ± 0.14	173.77 ± 22.72
M. aeruginosa	1.1	137.56 ± 0.16	229.95 ± 23.68
P. boryanum	1.1	137.90 ± 0.04	279.07 ± 23.95
M. aeruginosa	7.1	137.46 ± 0.15	186.31 ± 22.12
P. boryanum	7.1	137.94 ± 0.03	209.39 ± 16.51
M. aeruginosa	1.1	192.93 ± 0.03	248.54 ± 51.03
P. boryanum	1.1	195.01 ± 0.30	353.17 ± 29.54
M. aeruginosa	7.1	192.89 ± 0.01	216.89 ± 18.46
P. boryanum	7.1	194.56 ± 0.08	298.54 ± 28.71

carp may be by mucus entrapment as suggested for tilapia by several authors (Greenwood, 1953; Northcott and Beveridge, 1988; Beveridge *et al.*, 1989).

Ingestion rates per g body weight of fish per hour for smaller fish were observed to be higher than for larger fish in all trials (Table 4.5) although ingestion rates per fish were observed to be higher in larger than smaller fish. This may be because smaller fish need more food per g body weight than larger fish to sustain rapid growth. Energy loss as heat through the body surface is higher in smaller fishes than among larger fishes, in part explaining why smaller fishes require more food in per g body weight terms (Steffens, 1989; Hepher, 1988). Kato *et al* (1982) observed that the filtering rate of the copepod *Euphausia superba* Dana increased with body size, although filtering rates per g body weight decreased with increasing body size.

The results also show a significant reduction in mean *P. boryanum* colony diameter during the feeding period in some treatments (Table 4.2, 4.3 and 4.4). Analysis of size frequency distributions indicate slight reductions in trials with higher initial colony densities during feeding trials with rohu and catla. One explanation is that rohu and catla selectively feed on *P. boryanum* colonies > 65 µm diameter. The fry and fingerlings of rohu and catla selectively feed on zooplankton and on some selected phytoplankton in natural environment (Khan and Siddiqui, 1973; Jafri and Mustafa, 1977; Jhingran and Pullin, 1985; Pillay, 1990). Size selection in filter feeding is a widely reported phenomenon (Porter, 1977; Ishii *et al.*, 1985, Smith, 1989;

Hoogenboezem *et al.*, 1991).

Not only is there evidence of size selection and species selection but also the abilities among filter feeding carps to distinguish between toxic and non-toxic strains of *M. aeruginosa*. Grazing by silver carp was almost completely suppressed in the presence of the toxic stain of *M. aeruginosa*. Cyanobacteria are exploited to only a limited degree by zooplankton because of problems of manageability (cell/colony size), nutritional quality and toxicity (de Bernardi and Giussani, 1990; DeMott and Moxter, 1991). Concerning manageability, although silver carp is capable of entrapping and ingesting particle as small as unattached bacteria (< 1 μm diameter) from the water column (Chapter IV), it is undoubtedly more efficient at feeding on larger, colonial algal species than on small, unicellular organisms (Adamek and Spitler, 1984). Silver carp, however, can and do ingest *M. aeruginosa* (Spataru and Gophen, 1985). Compared with diatoms and other phytoplankton, Cyanobacteria have high levels of protein, in excess of 50% on a dry weight basis (Cummins and Wuychek, 1971; de Moor and Scott, 1985), although there is some evidence that *M. aeruginosa* is poorly digested by the stomachless silver carp and that conversion efficiencies are low (Hamada *et al.*, 1983; Bitterlich, 1985). The present results suggest that in the presence of toxic blooms of *M. aeruginosa*, ingestion by silver carp, and hence growth, are suppressed.

Although increased opercular beat rates, indicating feeding activity, were observed in

fish exposed to the non-toxic strain of *M. aeruginosa*, general activity levels of animals held in toxic Cyanobacteria were much lower than those of the control or non-toxic treatments. Similar responses have been found in other studies of fish exposed to planktonic food (Beveridge *et al.*, 1988, 1991), as well as to toxic and non-toxic irritants such as formalin and suspended solids (Ross *et al.*, 1985). Present results, in which opercular beat rates of fish exposed to the toxic strain did not differ significantly from the control (fish alone), show that fish react to toxic *M. aeruginosa* in a different manner from food and irritants. The feeding mechanism of silver carp has been little studied. Ingestion of the non-toxic strain of *M. aeruginosa* in this study may have been by mucus entrapment. However , if , as has been suggested elsewhere (Greenwood, 1953; Northcott and Beveridge, 1988), filter feeding by tilapias involves entrapment by mucus in the gill apparatus, a mechanism that almost certainly cannot be rapidly switched off or on, then toxic cells must also be retained by the fish. The implication here is that toxic cells can be detected and although perhaps trapped by the buccopharyngeal apparatus are rejected as food items.

Analysis of water samples by HPLC confirmed that no detectable levels of microcystin (<250 ng l^{-1}) were present in the water and that the observed responses were to whole cells. Results of Chapter III showed that the buccopharyngeal cavity of silver carp is heavily populated with taste buds and although the precise function of these remains unknown, it is probable that they play an important role in assessing the palatability of food items. Discrimination between toxic and non-toxic *M. aeruginosa* would seem

to be on the basis of very low levels of microcystin (< 250 ng l^{-1}) and/or other differences between toxic and non-toxic cells. The selection of toxic and non-toxic Cyanobacteria of similar cell size in the present study eliminates particle size as the basis for discrimination in grazing. Whether other differences, for example in cell surface components or olfactory compounds, could account for this is unknown.

CHAPTER V

Filter-feeding of carps on bacteria

5.1. Introduction

The role of bacteria in the diets of fish and their importance in aquatic production is not yet clear (Kuznetsov, 1977; Moriarty and Pullin,1987). Although both free-living and bacteria attatched to algae, suspended particles and organic matter occur in aquatic ecosystems, it is generally presumed that fish such as tilapias and carps can ingest only the attatched bacteria (Bowen,1976; Schroeder, 1978; Opuzynski, 1981b). In standing natural freshwaters most of the bacteria are free-living, total numbers of bacteria varing with the available suspended particles and season of the year (Kirchman, 1983; Pedros-Alio and Brock, 1983). Consumption and assimilation of films of laboratory cultures of methane-oxidizing bacteria by larval silver carp and bighead carp has been reported (Panov *et al.*,1969, cited by Kuznetsov, 1977). Filter feeding carps such as silver carp may also be able to ingest bacteria associated with algae (Kuznetsov, 1977). However there is no published information whether silver carp, rohu and catla can detect or ingest unattatched bacteria. Beveridge *et al.*(1989) found that *Oreochromis niloticus* fry were able to detect unattatched bacteria and there was a significant, positive correlation between bacterial ingestion and their concentration in the trial media.

Beveridge *et al.* (1991) observed that *Cyprinus carpio* were also able to detect unattached bacteria.

The present experiment was undertaken to establish whether silver carp, rohu and catla could detect and ingest unattached suspended bacteria.

5.2 Materials and Methods:

5.2.1 Fish

H. molitrix, L. rohita and C. catla fry, weighing 1-2 g were selected from the stocks maintained in the tropical aquarium, as described in Chapter III.

5.2.2 Bacteria

The bacterium *Chromobacterium violaceum* was first described by Bergonzini in 1981 (see Sneath, 1984). *C. violaceum* occurs mainly in soil and water and is common in tropical countries. It occasionally causes serious pyogenic or septicemic infections of mammals, including man (Sneath, *ibid*). It is a gram-negative, rod-shaped bacterium and typically measures 0.6 - 0.9 x 1.5 - 3.5 µm, with rounded ends, sometimes slightly curved (Sneath, 1984). The species is characterised by the presence of a violet pigment called violacein which is soluble in alcohol, and is readily apparent when the colonies are several days old. The colonies grow well on ordinary media, giving cream or

yellowish colonies that turn purple at the edges (Fig. 5.1). *C. violaceum* is mesophilic, growing at 37°C and the optimum temperature is 30-35°C. They can grow within 2-3 days in incubators at 22°C (Sneath, 1984). The bacterium has the advantage that because of its violet pigmentation it can be readily identified in the presence of other bacteria. The bacterial strain used, originated from NCIMB (National Collection of Industrial and Marine Bacteria Ltd, Aberdeen, U.K.), and was obtained from stock cultures held in the Bacteriology Laboratory at the Institute of Aquaculture, University of Stirling, U.K. The stock cultures were sub-cultured in Tryptone Soya Agar (TSA) medium to obtain pure colony growths. Sub-culturing was carried out twice a week to maintain the bacteria in its log phase of growth.

5.2.3 Preparation of agar media and inoculation

Tryptone Soya Agar (TSA, Oxiod) was used throughout the experiment as the plating and counting medium. Standard microbiological methods were used throughout (Collins and Lyne, 1976). Twenty grammes of TSA powder were mixed with 500 ml of distilled water in a glass bottle and shaken vigorously to dissolve. The solution was then sterilised by autoclaving at 121°C for 15 min and allowed to cool to about 45°C in a heated water bath in order to keep the agar in a molten phase. Some 15-20 ml of the molten agar medium was poured into each sterile petri-dish, the lid replaced, and the dish left undisturbed until the agar set. Before use, the freshly made agar plates were dried by placing them in an incubator set at 37°C for 15-20 min, with the lids

partly open. The streak plate technique was used for the inoculation of agar plates. All inoculated plates were properly labelled and incubated at 22°C for 2-3 days. In all cases care was taken to avoid contamination.

5.2.4 Experimental system.

Three tanks of size 44 x 24 x 26 cm, each containing 2 experimental chambers of 2 l capacity, were established in an isolated area in the tropical aquarium. The experimental chambers were cleaned and filled with 1 l of sterilised (autoclaved) tap water and placed in the tanks each of whose sides was covered with black polythene to minimise disturbance. Each experimental chamber was fitted with an autoclaved air hose and air stone. In order to maintain the water temperature in the experimental chamber at 27°C±1°C, water from a header tank fitted with a thermostatically-controlled heater was passed continuously through the tanks. The tops of the experimental chambers were covered with a sheet of transparent perspex and illumination was by natural, incident light.

5.2.5 Preliminary investigation of carp alimentary canal for *C. violaceum*

An investigation was made to establish whether *C. violaceum* was present in the alimentary canal of the experimental fish. At the beginning of the experiment, the fish were transferred from holding tanks in the recycle system of the tropical aquarium into

an isolated tank within the system where they were starved for 24 h. A group of 5 starved fish was then transferred to an experimental chamber. A replicate treatment for each species of fish was carried out. After 4 h acclimatization in the experimental chamber, all fish were killed in order to examine the intestinal contents.

Having killed the fish, the body cavity was immediately opened, the entire intestine removed as quickly as possible, chopped into small pieces, and the contents carefully flushed out into individual sterile test tubes and made up to 10 ml with physiological saline. They were then mixed for 3 min by a vortex mixer. Using a micropipette 0.1 ml of sample was then spread out on the agar plates by a glass spreader and the plates incubated for 3 days at 22°C in order to test for the growth of *C. violaceum*.

5.2.6 Ingestion experiment

Prior to the start of each trial, single species groups of five experimental fish were removed from the stock tanks in the recycle system, weighed and moved to another tank within the system where they were kept in isolation without feeding for 24 h. The single-species groups were then transferred to the experimental chambers and allowed 4 h to acclimatize.

C. Violaceum were transferred from a culture plate to a sterile bottle containing 2 ml physiological saline (pH 6.6), agitated for 2 min in a vortex mixer to ensure no clumps of bacteria were present, and added to the experimental tank drop by drop thus minimising disturbance to the fish. The suspension was rapidly dispersed by aeration. After 1 min a 5 ml sample was removed from the tank and diluted in the same way as the intestinal samples (see below) to determine the initial bacterial count.

At the end of each trial, all five fish in each tank were sacrificed. The entire intestine was removed as quickly as possible, chopped into small pieces and the contents carefully flushed out, transferred to a sterile test tube and made up to 10 ml with physiological saline and then mixed thoroughly with a vortex mixture. Using a micropipette, 1 ml of the mixed intestinal contents was transferred to a fresh sterile tube containing 9 ml buffered saline and the contents again mixed thoroughly. Five serial 1: 10 dilutions were prepared in the same way from each intestinal sample. Samples of 0.1 ml were then removed from each dilution and spread on agar plates. Duplicate plates were made in all cases. Plates were incubated at 22°C for 72 h.

For counting purposes, only plates containing between 3 and 80 colonies were selected. By multiplying the number of colonies by the reciprocal of the dilution, colony numbers per unit sample volume were determined.

Five trials were carried out for each species of fish in which groups of five fish were exposed to bacteria. In the first series of experiments the abilities of fry to ingest bacteria and the effects of exposure time (30 min, 1 h, 2 h and 4 h) on ingestion were examined. In a second set of experiments, the effects of bacterial concentration on ingestion rate, in which carp were exposed to different bacterial concentrations for a period of 1 h, were examined.

Statistical analysis was by Student's t-test (Bailey, 1981).

5.3. Results

5.3.1. Effects of exposure time on the ingestion of bacteria

The initial concentrations of bacteria established in the experimental chambers in the trials ranged from 3.2×10^5 to 27.3×10^5 cells ml^{-1}. During dissection of the carp stomachs at the end of the exposure period, the contents of some of the fish were observed to be stained a deep purple, characteristic of the bacterial pigment. The proportion of fish ingesting bacteria and the mean viable colony counts per fish for each trial are summarized in Table 5.1. The proportion of fish ingesting bacteria and the numbers of viable bacteria isolated from the alimentary tracts increased with time.

Table 5.1 Ingestion of bacteria by silver carp, rohu and catla; number of fish ingesting bacteria and number of ingested viable bacteria per fish at different exposure times.

Mean wt. of fish(g)	Initial bacterial concentration (cells ml^{-1})	Exposure time (h)	Mean number of viable cell (fish gut^{-1})	Number of fish (%) ingesting bacteria
1.1S	$3.2x10^{5}$	0.5	1500	1 (25%)
1.2S	$3.2x10^{5}$	1.0	4000	2 (50%)
1.1S	$3.2x10^{5}$	2.0	12500	3 (75%)
1.2S	$3.2x10^{5}$	4.0	16000	4 (100%)
1.2S	$12.3x10^{5}$	0.5	3500	2 (50%)
1.1S	$12.3x10^{5}$	1.0	12000	3 (75%)
1.1S	$12.3x10^{5}$	2.0	24500	4 (100%)
1.2S	$12.3x10^{5}$	4.0	36500	4 (100%)
1.2S	$24.6x10^{5}$	0.5	8000	2 (50%)
1.1S	$24.6x10^{5}$	1.0	20500	4 (100%)
1.2S	$24.6x10^{5}$	2.0	35500	4 (100%)
1.1S	$24.6x10^{5}$	4.0	68500	4 (100%)
1.5R	$3.4x10^{5}$	0.5	1200	1 (25%)
1.8R	$3.4x10^{5}$	1.0	3000	2 (50%)
1.5R	$3.4x10^{5}$	2.0	6500	2 (50%)
1.6R	$3.4x10^{5}$	4.0	11500	3 (75%)
1.7R	$10.2x10^{5}$	0.5	3500	1 (25%)
1.5R	$10.2x10^{5}$	1.0	5000	2 (50%)
1.6R	$10.2x10^{5}$	2.0	12000	3 (75%)
1.6R	$10.2x10^{5}$	4.0	18500	4 (100%)
1.7R	$27.3x10^{5}$	0.5	7500	1 (25%)
1.5R	$27.3x10^{5}$	1.0	13500	2 (50%)
1.8R	$27.3x10^{5}$	2.0	28000	2 (50%)
1.5R	$27.3x10^{5}$	4.0	40500	3 (75%)
1.5C	$3.8x10^{5}$	0.5	000	0
1.3C	$3.8x10^{5}$	1.0	300	1 (25%)
1.3C	$3.8x10^{5}$	2.0	500	2 (50%)
1.5C	$3.8x10^{5}$	4.0	1100	2 (50%)
1.4C	$11.3x10^{5}$	0.5	200	1 (25%)
1.5C	$11.3x10^{5}$	1.0	500	1 (25%)
1.3C	$11.3x10^{5}$	2.0	900	2 (50%)
1.4C	$11.3x10^{5}$	4.0	1600	2 (50%)
1.5C	$25.6x10^{5}$	0.5	700	1 (25%)
1.3C	$25.6x10^{5}$	1.0	1400	2 (50%)
1.4C	$25.6x10^{5}$	2.0	2500	2 (50%)
1.4C	$25.6x10^{5}$	4.0	6500	3 (75%)
1.3B	$3.5x10^{5}$	0.5	1800	2 (50%)
1.2B	$3.5x10^{5}$	1.0	4500	3 (75%)
1.2B	$3.5x10^{5}$	2.0	10500	4 (100%)
1.1B	$3.5x10^{5}$	4.0	16500	4 (100%)
1.2B	$11.6x10^{5}$	0.5	5500	1 (25%)
1.1B	$11.6x10^{5}$	1.0	13000	3 (75%)
1.1B	$11.6x10^{5}$	2.0	23000	4 (100%)
1.3B	$11.6x10^{5}$	4.0	37500	4 (100%)
1.1B	$25.1x10^{5}$	0.5	7500	3 (75%)
1.2B	$25.1x10^{5}$	1.0	22500	4 (100%)
1.0B	$25.1x10^{5}$	2.0	40000	4 (100%)
1.2B	$25.1x10^{5}$	4.0	71000	4 (100%)

* S = Silver carp; R = Rohu; C = Catla and B = Bighead carp.

5.3.2.Effects of bacterial concentration on ingestion rate

In the second series of experiments, groups of carp fry were exposed to concentrations of bacteria ranging from 4.2 x 10^5 to 27.6 x 10^5 cells ml^{-1}. The numbers of viable bacteria isolated from the fish were plotted against bacterial concentration in the experimental chambers in Fig. 5.1. Highly significant positive correlations ($P < 0.05$) were observed. The ingestion of bacteria appeared to increase with concentration in the media and the numbers of viable bacterial colonies isolated from silver carp and bighead carp intestinal contents were observed to be the highest whilst those isolated from catla were lowest (Figure 5.1).

5.4. Discussion

The results indicate that planktivorous carp fry are able to ingest unattached bacteria. Sonication of bacteria ensured that no clumps remained prior to introduction to the experimental tanks, and although it is posible that mucus sloughed from the fish skin may have caused clumping of bacteria, thereby faciliting ingestion, there was no visual evidence to support this supposition. The proportions of fish ingesting bacteria and the numbers of bacteria ingested increase with time and with bacterial concentration in the media. These findings corroborate those of Beveridge *et al.* (1989, 1991) for common carp and tilapia fry.

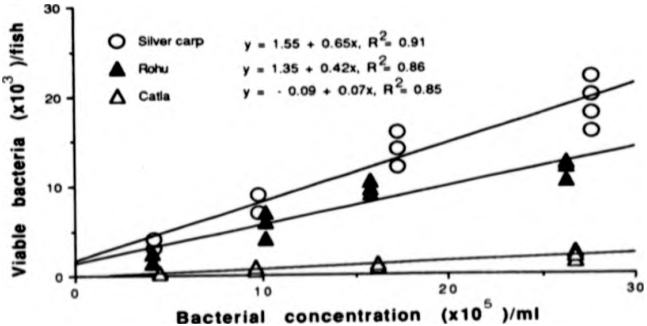


Fig. 5.1 The relationship between mean viable bacterial counts isolated from intestinal tracts of silver carp, rohu and catla plotted against initial bacterial concentration in tank water.

The mechanisms involved in ingestion of unattached bacteria by fish remain unresolved. The mesh sizes of the branchial sieve of silver carp, rohu and catla are around 20-25 μm , 75-90 μm and 110-130 μm respectively (see Chapter III) and are clearly unsuited to entrapment of bacteria if regarded solely as passive sieving devices. Studies of silver carp have demonstrated that it can not ingest particle size less than 5 μm by filter feeding (Adamek and Spittler, 1985; Smith, 1989). However, Kuznetsov (1977) stated that silver carp ingest aggregates of bacteria associated with algae and speculated that it may be able to ingest solitary bacteria by entrapment in mucus secreted by the labyrinthiform organ. Studies of filter-feeding in tilapias show that particles are entrapped among the gill apparatus in a mucus film (Greenwood, 1953; Drenner *et al.*, 1987; Northcott and Beveridge, 1988; Beveridge *et al.*, 1988a, b). The mucus is highly negatively-charged and it has been suggested that it may facilitate flocculation and hence entrapment of very small particles such as bacteria (Northcott & Beveridge, 1988; Beveridge *et al.*, 1989). Moreover, a recent paper by Sanderson *et al.* (1991) suggests that mucus-assisted entrapment of particles on the roof of the oral cavity may be important in some suspension-feeding cyprinids.

An alternative means of ingestion of bacteria may be through drinking. Although freshwater fishes drink little water (Evans, 1984), since they are continuously faced with the need to dispose of water which enters by osmosis across the gills and skin, the quantities that would be needed to explain the bacterial numbers found in the guts of small carps are small. Data summarised in Table 5.2 suggests that volumes < 40 μl

Table 5.2 Numbers of fish ingesting bacteria and rates of ingestion observed in groups of silver carp, rohu and catla held at different bacterial concentrations during an 1 h period.

Species of fish	Mean weight of fish (g)	No. of cells added per ml of experimental tank water	Mean number of cells ingested per fish	Number (%) of fish ingesting bacteria in each trial
H. molitrix	1.1	4.3×10^6	3,500	2 (40%)
L. rohita	1.6	4.2×10^6	2,000	2 (40%)
C. catla	1.4	4.6×10^6	300	1 (20%)
A. nobilis	1.2	4.3×10^6	4,000	2 (40%)
H. molitrix	1.1	9.8×10^6	8,000	2 (40%)
L. rohita	1.6	10.2×10^6	5,000	3 (60%)
C. catla	1.4	9.6×10^6	700	1 (20%)
A. nobilis	1.2	9.7×10^6	8,500	4 (80%)
H. molitrix	1.1	17.3×10^6	14,500	3 (60%)
L. rohita	1.6	15.8×10^6	7,500	4 (80%)
C. catla	1.4	16.2×10^6	1,000	2 (40%)
A. nobilis	1.2	17.3×10^6	15,000	5 100%)
H. molitrix	1.1	27.6×10^6	20,500	4 (80%)
L. rohita	1.6	26.3×10^6	12,500	4 (80%)
C. catla	1.4	26.8×10^6	2,000	2 (40%)
A. nobilis	1.2	27.5×10^6	21,500	5 (100%)

h^{-1} may be involved. This can be accounted for by estimated drinking rates in freshwater teleosts (1-182 $\mu l\ h^{-1}$) (Potts et al., 1967; Eddy and Bath, 1979). However, the number of bacteria ingested by tilapias cannot be explained by drinking alone.

In this experiment the highest number of viable bacteria were isolated from silver carp and the lowest from the catla. Silver carp may indeed ingest more bacteria than other carps although the results may to some extent reflect interspecific differences in conditions in the intestinal tract or differences in environment in the buccopharyngeal region. It is clear from published data on *C. violaceum* ingestion by tilapia (Beveridge *et al.*, 1989) that many fewer bacteria have been isolated from carp than tilapia guts although bacterial concentrations in the water and exposure times were similar. However, because of interspecific differences discussed above, it is not possible to conclude if carps ingest less unattached bacteria than tilapia. On the basis of numbers isolated from gut contents and low mass of individual bactrial cells, however, it is unlikely that unattached bacteria play a significant role in fish nutrition in either gross energy or trace element terms.

CHAPTER VI

General Discussion

In the present study an attempt was made to investigate the possible filter feeding mechanisms with respect to the feeding ecology of three Asian carps, *L. rohita*, *C. catla* and *H. molitrix* through detailed study of the bucco-pharyngeal apparatus and experimental studies of quantitative feeding behaviour on algae and bacteria. Due to limited facilities it was not possible to measure the extent of gill raker adjustment in relation to the size of food particles. It was also impossible to study the flow of water through the mouth, the movement of water inside the bucco-pharyngeal cavity, intensity of mucus cell production from the bucco-pharyngeal cavity, or the movement of the surface of the palatal organ during feeding. Such studies are desirable in order to complete our understanding of how filter-feeding is achieved.

Nevertheless, on the basis of observations made during the present study a tentative description of filter-feeding is possible. Rohu, catla and silver carp may be described as pump filter-feeders (*sensu stricto* Lazzaro, 1987), taking water in through their mouths and passing it through the branchial sieve and out through the gill opercular openings during both feeding and respiration. The sequence of events is as follows: during feeding, fish were observed to take water in through the mouth whilst keeping the opercular opening closed. Water was drawn in by expanding the bucco-pharyngeal

region as a result of lowering of the floor of the region and widening of the lateral bucco-pharyngeal region sides. The mouth was then closed, the operculae opened and the bucco-pharyngeal region compressed in order to pump the water through the branchial sieve into the opercular cavity and from there out through the opercular openings. The respiratory valve at the base of the upper lip (Plate 1a-c) prevented the water flowing back out of the mouth. The presence of folds on the buccal floor (Plate 2a-c) and lateral sides of the region of these three species lends support to the hypothesis of water inhalation by expansion of the region. However, although the basic principle of water intake and ejection appears to be the same for all three species, the mechanism of particle retention may well differ.

The retention of food particles in the branchial sieve depends on several factors: i) the mesh-size and homogeneity of the branchial sieve (Wright *et al.*, 1983; Drenner *et al.*, 1984; Gibson, 1988); ii) the size and shape of the food particles (Wright *et al.*, 1983; Gibson, 1988); iii) the orientation of the food particle when approaching the mesh of the branchial sieve (Hoogenboezem, 1991); iv) the surface chemistry of the branchial sieve (Northcott and Beveridge, 1988).

In the present study it was found that the branchial sieves of rohu, catla and silver carp were more-or-less homogeneous except for the posterior region of the gill arch channels. Moreover, the gill arch channels of rohu and catla are occluded by the palatal organ whilst those of silver carp penetrated into the suprabranchial organ. The

implications of these differences are discussed below.

It is virtually impossible to determine the orientation of approaching particles. Thus, it is generally accepted that a particle has a retention probability rather than an absolute retention value. The concept of retention probability is related to particle size and shape and possible orientation. Nevertheless, Wright *et al.* (1983) and Gibson (1988) both expressed difficulty in determining an appropriate measure of prey items which would reliably estimate retention probability because of questions of prey orientation in the bucco-pharyngeal cavity.

Results on algal feeding indicate that all three species were able to ingest *P. boryanum* colonies of 60 µm in diameter although only rohu and silver carp were able to ingest the much smaller *M. aeruginosa* cells (~ 4 µm diameter). The inter-gill raker spaces of rohu, catla and silver carp are 80-90, 110-120 and 25-30 µm respectively. It is thus clear that passive branchial sieving is not the only mechanism of particle retention in filter feeding among these carps.

The role of mucus in the filter feeding process of fishes has been proposed by several authors and most consider mucus is important for retention of food particles. Greenwood (1953) observed a discrepancy between the coarse architecture of the branchial sieve and the tiny phytoplankton ingested by *Tilapia esculenta* and suggested that these small particles are entangled in a copious mucus supply secreted by cells in

the epithelium of the bucco-pharyngeal cavity. Northcott and Beveridge (1988) and Northcott *et al.*, (1991) assumed that in the tilapia *Oreochromis niloticus*, mucus forms a net-like structure between the arches and this net helps in retention of *M. aeruginosa*. Beveridge *et al.*, (1989) proposed that mucus was responsible for entrapment of the bacterium *Chromobacterium violaceum* in *O. niloticus*. Mucus entrapment of food particles in cyprinids has also been reported by Sibbing and Uribe (1985) and Hoogenboezem (1991). The results of histological and ultrastructural examination in the present study show the presence of very large numbers of two types of mucus cells in the bucco-pharyngeal region of the carps studied. It is thus possible that mucus plays an important role in the retention of *M. aeruginosa* by rohu and silver carp.

The results from the experiments conducted on bacterial ingestion showed bacteria are ingested in comparatively small numbers (Table 6.1). There is no need to invoke the involvement of only mucus-based mechanisms, as ingestion by drinking would account for the small numbers found in the fore-gut. In view of the numbers involved Beveridge *et al.*, (1991) also suggested that the benthic-feeding common carp *Cyprinus carpio* ingest unattached suspended bacteria along with the drinking water, despite the fact that Sibbing and Uribe (1985) have proposed that the fish ingests small food particles by mucus entrapment. By contrast the tilapia *O. niloticus* ingests unattached suspended bacteria in considerable numbers (Table 6.1) and Beveridge *et al* (1989) proposed that mucus entrapment must be involved. Thus, from the present study and

Table 6.1 Summary of laboratory studies on ingestion of the bacterium *C. violaceum* by planktivorous fishes. Duration of trials in all cases is 0.5 h, except for *O. niloticus* (0.25 h).

Species	Size (g)	Bacterial concentration in media (x 10^6 ml^{-1}	Bacterial concentration in gut (x 10^3)	Reference
O.niloticus	2.6 - 3.4	2.2 - 14.0	500 - 17,000	Beveridge *et al* 1989
C. carpio	8.0 - 15.9	5.4 - 40.3	0.5 - 90	Beveridge *et al* 1991
	63.6 - 69.5	2.8 - 46.6	0.1 - 0.8	" " "
H. molitrix	1.1 - 1.2	3.2 - 24.8	1.5 - 8.0	Chapter V
L. rohita	1.5 - 1.8	3.4 - 27.3	1.2 - 7.5	"
C. catla	1.3 - 1.5	3.8 - 25.6	0 - 0.7	"

the review of literature on bacterial ingestion by fish, it may be concluded that tilapia ingest unattached suspended bacteria in significant numbers whilst carps do not.

The results from trials investigating ingestion of toxic algae showed that silver carp do not ingest toxic *M. aeruginosa*. Fish may detect the toxic algae in the environment by the numerous taste buds on their bucco-pharyngeal surface and either avoid ingestion it or reject them as a food item. Cyanobacteria (blue-green algae) often dominate the phytoplankton communities of eutrophic water bodies (Reynolds, 1984), including traditional and modern fisheries. Although many of these Cyanobacteria can produce powerful hepato- and neurotoxins which can kill if ingested in sufficient quantity (Skulberg et al., 1984; Codd et al., 1989), they are apparently an important component of the diet of a number of tropical cichlids and cyprinids (Bowen, 1982; Hofer and Scheimer, 1983; Adamek and Spittler, 1984; Spataru and Gophen, 1985). In the light of the present study, it must be concluded that fish may face food scarcity in many of these eutrophic environments where toxic algae may predominate, hampering fish growth and, if the toxins are liberated as the result of a bloom dying off, even causing mortality.

The results of the feeding experiments on algae at different densities indicate that ingestion rate changed with algal density. Similar results have also been reported by other authors (Seale and Wassersug, 1979; KÏrboe *et al.*, 1985; Northcott *et al.*, 1991).

From the present study and the above review of small food particle ingestion by carps and tilapias we may concur with the views expressed by other researchers (see above) and conclude that retention of small food particles depends on several factors: i) particle size; ii) particle density; iii) surface chemistry; iv) digestibility and v) energy content. Considering all these factors filter feeding mechanisms among carps are proposed as follows:

Rohu take in water along with particles into the bucco- pharyngeal cavity through the mouth. There are numerous taste buds on the bucco-pharyngeal surface which help the fish to detect and assess the particle and decide how it should be handled. With regard to handling food particles the animal may have three options:

i) if the food particle is sufficiently large to be passively entrapped by the branchial sieve fish can ingest it easily. This would be the least expensive means of particle retention in terms of energy expenditure.

ii) if a food particle is insufficiently large for passive retention by the branchial sieve it might still be retained by adjustment of the branchial sieve. Fish could thus ingest particles somewhat smaller than gill raker spacings might suggest, although this method is likely to be more expensive than the previously proposed mechanism in terms of net energy expenditure. Ingestion of *P. boryanum* by rohu can be explained by branchial sieve adjustment.

iii) if the food particle is insufficiently large to be trapped by passive means by the branchial sieve and even insufficiently large to be actively caught by readjustment of the branchial sieve members, it could still be utilized as food if the fish were able to use a mucus entrapment mechanism. In this mode of food particle retention fish use the gill arches not as a sieve, but as a barrier in order to create turbulent flow conditions inside the bucco-pharyngeal cavity. Under such conditions small food particles may then be entrapped by mucus produced from cells on the bucco-pharyngeal surface. Similar feeding mechanisms have also been proposed by Sanderson *et al.* (1991) for the cyprinid *Orthodon microlepidotus*. Ingestion of small *M. aeruginosa* cells (~ 4 µm diameter) by rohu with 80-90 µm gill raker spacings may thus be possible by mucus entrapment. Mucus entrapment, however, is likely to be the most expensive feeding mechanism for fish in view of costs of synthesis. However, fish ingest most of the mucus used for particle entrapment and, provided that it may be efficiently digested, this would compensate to some extent for the energy costs associated with this method of feeding.

Catla are likely to have somewhat different feeding methods and abilities from rohu. They have long free gill rakers with large inter-raker spacings (110-120 µm). Catla feed on both zooplankton and phytoplankton although they show positive selection preference for zooplankton (Jafri and Mustafa, 1977; Jhingran and Pullin, 1985; Pillay, 1990). Catla may use similar branchial sieving mechanisms to rohu for particle retention although in the present trials catla did not ingest *M. aeruginosa*. There are

a number of explanations: catla may reject the alga as a food item, or it may be unable to digest it or it may be suitable as a food but unprofitable to feed on such small phytoplankton in view of energy costs (see above).

In view of the comparatively small gill raker spacings (25-30 µm) passive branchial sieving is likely to be the principal mechanism used by silver carp for retention of particles larger than 20 µm although mucus is probably involved in entrapment of smaller food particles. Unlike the other two species silver carp have a suprabranchial organ on the roof of the pharyngeal cavity inside the palatal organ. The results of the histological study indicate the presence of large numbers of mucus cells on the walls of the suprabranchial organ. Each gill arch bears two rows of gill rakers which form channels, the posterior section of which enter into the suprabranchial organ. After drawing water containing food particles into the bucco-pharyngeal cavity, pressure is created which forces the water out through the branchial sieve. Silver carp have numerous taste buds on the bucco-pharyngeal surface which help the fish detect and assess the food particle. On the basis of particle size and other factors, perhaps related to olfactory properties, fish may adopt different strategies to retain those particles, such as:

i) if the food particles are large enough to be retained by the branchial sieve (> 20 µm), the fish may arrange the gill arch channels in such a way that the bulk of the water passes through the branchial sieve. The remaining water containing food particles

would enter the suprabranchial organ where food particles could be entraped by the posterior part of the organ. A bolus of food particles and mucus from several such pumping actions would then be passed back into the posterior part of the pharyngeal cavity and from there into the oesophagus by reversing the flow of water during contraction of the suprabranchial organ. Ingestion of large colonial *P. boryanum* by silver carp is likely to take place by passive branchial sieving.

ii) if the food particle is insufficiently large enough to be retained by the branchial sieve, the animal may arrange the gill arch channels in such a way that the water containing food particles is driven through the gill arch channels directly into the suprabranchial organ where the particles are trapped by the mucus cells and formed into a bolus. As a result of the pump action boluses of food plus mucus are passed back again into the pharyngeal cavity and into the oesophagus. It is proposed here that ingestion of small algal cells, such as *M. aeruginosa*, by silver carp occurs by this mechanism. The net energy expenditure by the fish is likely to be greater than the previous passive method of entrapment.

Dominance of larger food particles in the diet of silver carp has been reported by several authors (Boruckij, 1973 cited by Lazzaro 1987; Kajak et al., 1977; Adamek and Spittler, 1984). Boruckij (1973) also reported that silver carp feed selectively on particles larger than 20 µm. In the laboratory trials conducted in the present study the

ingestion rate of silver carp was higher when feeding on *P. boryanum* in comparison to *M. aeruginosa*.

From the present study of algal ingestion rates it was found that in all cases silver carp ingest highest amounts of algal biomass, followed by rohu and then by catla, although the ratio of total filtering area of the branchial sieve of rohu, catla and silver carp of same size is 1 : 2.78 : 3. Thus, despite having a greater filtering area than rohu, catla ingest less interms of algal biomass, indicating that the filtering apparatus of catla is less efficent in comparison with that of rohu or silver carp. In composite culture of Chinese and Indian major carp at higher densities, growth of Indian major carp such as catla and rohu are adversely affected (Lakshmanan *et al.*, 1971; Dey *et al.* 1979). Dewan *et al.* (1991) reported that the degree of dietary overlap among the fry and fingerlings of Chinese and Indian major carps is more prominent between catla and silver carp. In view of the information from the present studies it is recommended that Chinese carp should not be stocked together with Indian major carp, particularly with catla and particularly during the fry-fingerling stages.

On the basis of the results of Chapter IV, we may conclude that small (1-2 g) silver carp ingest highest amounts of algal bio-volume (in terms of g^{-1} body weight h^{-1}) when feeding at highest (193×10^4 μm^3) algal concentrations which are 9.32×10^9 - 6.56×10^9 μm^3 algae per day (calculated on the basis of 24 x the maximum hourly ingestion rate of *P. boryanum* determined from the feeding trials). Reynolds (1984) reported the

mass of *M. aeruginosa* as 32(23-44) pg cell^{-1}. Mean energy content of aquatic algae is 13.5 KJ g^{-1} dry weight (Cummins and Wuycheck, 1971). Hence it can be calculated that a small silver carp (1.1 g) might be able to ingest 0.11 - 0.15 KJ energy per day when only phytoplankton is avilable as food item. There is some evidence that *M. aeruginosa* and other phytoplankton are poorly digested by the stomachless silver carp and that conversion efficiencies are low (Kajak *et al.*, 1977; Hamada *et al.*, 1983; Bitterlich, 1985).

Brett and Groves (1979) derived a general energy budget for young, well-fed herbivorous fish :

$$100I = 37M + 20G + 43E$$

where, I = rate of ingestion, M = metabolic rate, G = growth rate, E = total excretion rate and E is further divided into fecal = 41 and non fecal = 2. All values are expressed in terms of percentage of total calories consumed (I). Chakraborty (1992) reported that *Cyprinus carpio* need 53.43 KJ/Kg/day energy to fulfill routine metabolic requirements. It is therefore clear that carp fry and fingerlings could not grow solely on a diet of planktonic algae, thereby explaining the obeservations that fry and fingerlings of rohu, catla and silver carp selectively feed on zooplankton (Khan and Siddiqui, 1973; Jaffri and Mustafa, 1977; Jhingran and Pullin, 1985; Pillay, 1990).

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APPENDIX- 1.

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

Constituent compound	Concentration in stock solution	Inclution in growth medium
$NaNO_3$	75.0g. 500ml^{-1}	10ml.l^{-1}
$MgSO_4$. $7H_2O$	15.0g. 200ml^{-1}	1ml.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 coprising		
H_3BO_3	2.680g.l^{-1}	
$MnCl_2$. $4H_2O$	1.810g.l^{-1}	
Na_2MoO_4. $2H_2O$	0.390g.l^{-1}	1ml.l^{-1}
$ZnSO_4$. $7H_2O$	0.22.g.l^{-1}	
$CuSO_4$. $5H_2O$	0.079g.l^{-1}	
$Co(NO)_2$. $6H_2O$	0.049g.l^{-1}	

APPENDIX- 2

Composition of JM (Jaworski's Medium) algal growth medium

Constituent compound	Concentration in stock solution	Inclusion in growth medium
$Ca(NO_3)_2$. $4H_2O$	4.0 g. 200 ml^{-1}	1 ml. l^{-1}
KH_2PO_4	2.48 g. 200 ml^{-1}	"
$MgSO_4.7H_2O$	10.0 g. 200 ml^{-1}	"
$NaHCO_3$	3.18 g. 200 ml^{-1}	"
EDTA FeNA EDTA Na_2	0.45 g. 200 ml^{-1} 0.45 g. 200 ml^{-1}	"
H_3BO_3 $MnCl_2$. $4H_2O$ $(NH_4)_6Mo_7O_{24}.4H_2O$	0.496 g 200 ml^{-1} 0.278 g 200 ml^{-1} 0.2 g 200 ml^{-1}	" " "
Cyanocobalamin Thiamine HCl Biotin	0.008 g 200 ml^{-1} 0.008 g 200 ml^{-1} 0.008 g 200 ml^{-1}	" " "
$NaNO_3$	16.0 g 200 ml^{-1}	"
Na_2HPO_4. $12H_2O$	7.2 g 200 ml^{-1}	"

APPENDIX- 3

Composition of BB (Bold's Basal Medium) algal growth medium

Contituent compound	Concentration in Stock solution	Inclusion in growth medium
$NaNO_3$	10.0 g / 400 ml	10 ml/l
$MgSO_4$. $7H_2O$	3.0 g / 400 ml	"
NaCl	1.0 g / 400 ml	"
K_2HPO_4	3.0 g / 400 ml	"
KH_2PO_4	7.0 g / 400 ml	"
$CaCl_2$. $2H_2O$	1.0 g / 400 ml	"
Trace element		
$ZnSO_4$. $7H_2O$	8.82 g / l	1 ml / l
$MnCl_2$. $4H_2O$	1.44 g / l	
MoO_3	0.71 g / l	
$CuSO_4$. $5H_2O$	1.57 g / l	
$CoNO_3$. $6H_2O$	0.49 g / l	
H_3BO_3	11.42 g / l	"
EDTA	50.0 g / l	"
KOH	31.0 g / l	
$FeSO_4$. $7H_2O$	4.98 g / l	"
H_2SO_4 (conc.)	1.0 ml / l	

APPENDIX-4

Haematoxylin -Eosin staining

(1) Bring section to water by bath in:	
Xylene	**5 min**
Absolute alcohol	**2 min**
Methylated spirit	**1.5 min**
(2) Haematoxylin	**10 min**
(3) Wash in tap water	**1 min**
(4) Differentiate in 1% acid alcohol	**4 quick drops**
(5) Wash in tap water	**1 min**
(6) Scott's tap water substitute	**1 min**
(7) Eosin	**3 min**
(8) Methylated spirit	**30 s**
(9) Absolute alcohol	**2 min**
(10) Absolute alcohol	**1.5 min**
(11) Xylene	**5 min**
(12) Mount in synthetic resin	

APPENDIX-5

Alcian blue staining at ph 2.5

Alcian blue stain: Alcian blue 0.5g
Glacial acetic acid 3 ml
Distilled water to 100 ml

(1) Bring section to water	
(2) Alcian blue stain	20 min
(3) Rinse in distilled water	
(4) Wash in running water	5 min
(5) Counter stain with 1% neutral red	
(6) Methylated spirit	30 s
(7) Absolute alcohol	2 min
(8) Absolute alcohol	1.5 min
(9) Xylene	5 min
(10) Mount in synthetic resin	

Observations: Acid mucopolysaccharide :green
Cell nuclei :red
Background :yellow

APPENDIX-6

Periodic Acid - Schiff's (PAS) Reaction

(1)	Sections to water	
(2)	1% Periodic acid	10 min
(3)	Wash in tap water	5 min
(4)	Schiff's reagent	20 min
(5)	Wash in tap water	
(6)	Haematoxylin	5 min
(7)	Wash in tap water	
(8)	Differentiate in 1% acid alcohol	
(9)	Blue in Scott's tap water substitue	
(10)	Wash in tap water	
(11)	Methylated spirit	30 s
(12)	Absolute alcohol	1 min
(13)	0.3% tartrazine in cellosive	3 min
(14)	Absolut alcohol	1.5 min
(15)	Xylene	5 min
(16)	Mount in synthetic resin	

Results : PAS positve red or megenta

Nuclei ; blue

APPENDIX- 7

Comparative gill morphomatric data of gill arch II and total filtering area of same size rohu, catla and silver carp.

	rohu	catla	silver carp
Standard Length (mm)	91	91	92
Weight (g)	17.52	19.53	15.33
Gill Raker Length (μm)	855	1575	1720
Gill Arch Length (μm)	13220	25430	24150
Gill Raker Space (μm)	95	130	30
Total Gill Raker no.	120	246	720
Total Taste Buds on Gill Arch II	>1850	>7000	>16000
Total filterin area (μm^2) x 10^7	5.83	16.22	17.63

APPENDIX - 8

Routine automatic process

1. 70 per cent alcohol	three hours
2. 90 per cent alcohol	three hours
3. Absolute alcohol I	one hour
4. Absolute alcohol II	one hour
5. Absolute alcohol III	two hours
6. Absolute alcohol IV	two hours
7. Toluene I	one and half hours
8. Toluene II	two and half hours
9. Wax bath I	three hours
10. Wax bath II	three hours
	22 hours

APPENDIX - 9

Colony numbers ± SD of *Pediastrum boryanum* at different concentrations of preservative at different duration of preservation.

Sample	BP	1 day	2 days	4 days	8 days	16 days	32 days	70 days
0.5% F.	1334 ± 4	1298 ± 12	1294 ± 6	1244 ± 4	1187 ± 7	1136 ± 5	1128 ± 3	1104 ± 4
1% F.	"	1284 ± 5	1277 ± 6	1233 ± 4	1225 ± 4	1200 ± 26	1108 ± 3	1092 ± 3
2% F.	"	1272 ± 4	1236 ± 4	1202 ± 2	1185 ± 5	1173 ± 4	1102 ± 3	1092 ± 5
4% F.	"	1237 ± 2	1221 ± 4	1193 ± 4	1182 ± 4	1172 ± 4	1092 ± 3	1083 ± 4
0.05% L.	"	1295 ± 6	1276 ± 6	1263 ± 3	1009 ± 6	925 ± 4	834 ± 5	643 ± 21
0.1% L.	"	1272 ± 4	1273 ± 3	1235 ± 3	1205 ± 4	1134 ± 4	1081 ± 4	1080 ± 3
0.2% L.	"	1267 ± 3	1261 ± 2	1236 ± 3	1205 ± 5	1131 ± 3	1083 ± 5	1074 ± 4
0.4% L.	"	1223 ± 6	1202 ± 2	1181 ± 4	1164 ± 5	1102 ± 3	1075 ± 3	1064 ± 2

*BP = Before Preservation; F. = Formalin; L. = Lugol's iodine.

APPENDIX - 10

Colony numbers ± SD of *Microcystis aeruginosa* at different concentrations of preservatives at different durations of preservation.

Sample	BP	1 day	2 days	4 days	8 days	16 days	32 days	70 days
0.5% F.	22439 ± 110	22265 ± 97	22141 ± 66	21938 ± 60	21284 ± 66	19948 ± 65	19407 ± 136	18127 ± 71
1% F.	"	22167 ± 77	21840 ± 54	21447 ± 109	20766 ± 67	19550 ± 64	19450 ± 77	18094 ± 41
2% F.	"	21746 ± 120	21356 ± 72	20970 ± 83	20514 ± 97	20150 ± 76	19338 ± 63	18023 ± 45
4% F.	"	21355 ± 53	21240 ± 70	20837 ± 57	20653 ± 73	20036 ± 64	19234 ± 63	17929 ± 50
0.05% L.	"	21868 ± 41	20247 ± 63	17506 ± 95	9706 ± 83	3476 ± 86	2084 ± 39	1528 ± 20
0.1% L.	"	22075 ± 83	20446 ± 51	20366 ± 50	19559 ± 47	16538 ± 72	16440 ± 65	16059 ± 67
0.2% L.	"	21746 ± 71	21430 ± 59	21252 ± 75	20757 ± 47	20334 ± 61	19729 ± 50	18407 ± 28
0.4% L.	"	21503 ± 93	21243 ± 59	21157 ± 72	20603 ± 96	20158 ± 75	19353 ± 65	17914 ± 26

BP = Before Preservation; F. = Formalin; L. = Lugol's Iodine.

APPENDIX - 11

Cell numbers ± SD of *Chlorella vulgaris* at different concentration of preservatives at different duration of preservation.

Sample	BP	1 day	2 days	4 days	8 days	16 days	32 days	70 days
0.5% F.	35366 ± 39	34439 ± 44	34144 ± 47	33662 ± 46	33164 ± 72	32568 ± 66	31747 ± 62	31245 ± 68
1% F.	"	34167 ± 34	33688 ± 84	33366 ± 58	32653 ± 52	31745 ± 57	31061 ± 91	30535 ± 50
2% F.	"	33477 ± 55	33261 ± 77	32754 ± 48	32340 ± 64	31560 ± 59	30939 ± 53	30147 ± 68
4% F.	"	33238 ± 52	32845 ± 62	32455 ± 57	31946 ± 52	31250 ± 42	30247 ± 44	29106 ± 47
0.05% L.	"	33927 ± 44	33893 ± 71	33682 ± 98	32461 ± 75	32146 ± 62	31565 ± 75	30929 ± 54
0.1% L.	"	33643 ± 57	33547 ± 50	33273 ± 74	32151 ± 63	31758 ± 60	30756 ± 74	30137 ± 74
0.2% L.	"	33559 ± 53	33253 ± 49	32755 ± 63	32035 ± 65	31546 ± 51	30234 ± 64	29843 ± 51
0.4% L.	"	33202 ± 27	32871 ± 57	32459 ± 61	31764 ± 67	31336 ± 69	30253 ± 44	29319 ± 71

* BP = Before Preservation; F. = Formalin: L. = Lugol's iodine