CHAPTER TWO

GENERAL MATERIAL AND METHODS
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2.1 Experimental animals:

(a) Collection and transportation of fish:

The indigenous predatory catfishes *Heteropneustes fossilis* (Bloch.) and *Mystus vittatus* (Bloch.) inhabit natural as well as artificial freshwater habitats of India. (Muddanna, 1971; David & Rao, 1976; Dehadral et al., 1977; Jhingran, 1982). A preliminary survey by Arunachalam (1978) and Reddy (personal communication) suggests their widespread occurrence in the freshwater habitats of Bangalore. During the present work, based on the abundance, these two species were collected regularly from two habitats located in the south taluk of Bangalore. Individuals of *H. fossilis* were collected from the Bennagannahalli tank (13° North latitude and 77° 40' East longitude), while those of *M. vittatus* were collected from the Gottegere tank (12° 13' North latitude and 77° 38' East longitude). Periodic fish collections were made using three types of gear:

1. Plough net (mesh size: 0.50 x 0.50 cm),
2. Cast net (diameter 4 m, mesh size: 10 mm) and
3. Drag net (mesh size: 5 mm).

Fish caught in these nets were brought to the shore and individuals of the required sizes were segregated. These individuals were then transported to the laboratory in plastic containers (height: 29 cm, diameter: 29 cm) in 10 l of freshwater. Since both the species possess sharp spines in the pectoral fins, injury to individuals during transportation was prevented by maintaining a density of only 20 fish per container.
(b) Maintenance of the laboratory stocks

On arrival at the laboratory, the fishes were given a quick dip (<30 sec) in a 0.1% Potassium permanganate solution to prevent dermal infection. Subsequently, fishes of similar size were maintained in large stocking tanks (61 x 31 x 33 cm) in 20 l of freshwater for seven days. During this period, the water was continuously aerated and the fishes were fed on an ad libitum diet of fresh muscles of the cyprinid fish, Rasbora daniconius (Ham.) and/or the oligochaete worm, Tubifex tubifex (Muller). A constant check was maintained on the condition of the fish, and dead or diseased individuals were removed and discarded. To prevent any kind of infection, 125 mg of Terramycin was also added to the water each stocking tank.

2.2 Holding systems and their management:

(a) Experimental holding systems:

All the feeding and growth experiments on the catfishes were conducted in glass aquaria (38 x 38 x 25 cm) containing 15 l of tested water. The aerator connections for these tanks were made serially and the water in each aquarium was aerated daily from 18.00** to 08.00** IST. Reduction in the level due to evaporation was corrected by the addition of the required quantity of the water. A daily photoperiod cycle of 10L : 14D (L:L 08.00** to 18.00** ; DD 10.00** to 08.00** IST) was maintained throughout the period of the experiments. The illumination was provided through cool fluorescent tubes. The intensity of light at the surface of water averaged to 769 lux. (Fig.3).
Figure 3

Experimental set up for laboratory growth studies of *Heteropneustes fossilis* and *Mystus vittatus*. 
Fig. 3
(b) Chemistry of water used for stocking and feeding experiments:

Many physico-chemical factors are known to influence the toxicity of biocides (Lloyd & Herbert 1962; Lloyd, 1963; Tarzwell, 1971; Rehwoldt et al. 1971). Several workers have suggested the maintenance of a constant level of temperature, pH, dissolved oxygen, hardness and alkalinity of the toxicant-free water prior to its treatment with toxic substances, as also during the course of the experiments, with the toxicants dissolved in it (see also Lee, 1973; Alabaster & Lloyd, 1980). During the present studies, the water used for maintenance of the stock fish, and that used for feeding experiments was analysed regularly for recording the temporal variations if any, of the following physico-chemical factors:

**Temperatures:** This was recorded using a centigrade thermometer.

**pHs:** pH of water was measured using a pH meter with a glass electrode. (Ellico; model CL44).

**Free Carbon dioxide:** This was estimated following the method detailed in APHA, AWWA, WPCF (1980) and the values were expressed as mg/l.

**Total alkalinity:** The carbonate and bicarbonate alkalinitites were determined by employing the method detailed in APHA, AWWA, WPCF (1980). The total alkalinity values were expressed as mg CaCO₃/l.

**Total hardness:** Total hardness was determined by the EDTA titrimetric method as given in APHA, AWWA, WPCF (1980) and was expressed as mg CaCO₃/l.

**Calcium:** The calcium content of water was determined titrimetrically using EDTA (APHA, AWWA, WPCF, 1980). The values were given as mg CaCO₃/l.

**Dissolved Oxygen:** The dissolved oxygen content of water was estimated titrimetrically by employing the modified Winkler's method (Lind, 1974) and the
results are expressed as mg/l.

The above physico-chemical analysis was also performed with water samples with different levels of the toxicants dissolved in them.

2.3 Experimental design for feeding and growth studies:

(a) Selection of experimental fishes:

Active individuals of the two species of catfishes, *Heteropneustes fossilis* and *Mystus vittatus* were selected from the laboratory stock based on their size. The two species of fishes were maintained separately in 15 l of the respective media. *H. fossilis* were reared at a density of 3 fish per aquarium and for *M. vittatus* the density used was 5 fish per aquarium. All the fishes were starved for 3 days in toxicant-free water (freshwater) prior to the initiation of the feeding schedule and the feeding experiments were conducted in a room, where apart from feeding, all other disturbances were kept at a minimum. A daily record of the water temperature was maintained for the entire duration of the experiments, and the temperature in the feeding room during the study period averaged 23.5 ± 2.0°C.

(b) Live and dry weight determinations:

Prior to the initiation of each feeding experiment, a group of 5 or 6 fish of either species, in the required size range were sacrificed to determine the initial live and dry weights. The live weight was determined after drying each fish by blotting on a filter paper, and the fish were further dried in a hot air oven at 60°C to constant weight. This group of fish served as the initial control.
At the end of each feeding experiment, live and dry weights of the experimental fish were also similarly determined.

(c) Experimental food and feeding procedures:

Fresh muscles of Cyprinid fish, Rasbora daniconius (Ham.) were used as food. R. daniconius were collected regularly from a local freshwater tank, stocked in the laboratory and utilized for feeding the catfishes as and when necessary.

For feeding, active individuals of R. daniconius were selected from the stock, decapitated and the scales were removed. The body muscles were then excised, cut into sizeable pieces, cleaned in water and used as the experimental feed. After blotting on a filter paper, the food was weighed accurately with an electric balance (Adayar Dutt, Model No: AD 100), and fed to the fish. A series of different ration levels was offered daily to H. fossilis and M. vittatus. The different levels of feeding offered were calculated as percentages of the initial live body weights of the respective fishes. The uningested food was collected the next day, blotted and weighed. Both feeding and collection of unfed were carried out daily between 12.00** and 14.00** IST. At each feeding level, 3 replicates were maintained.

2.4 Parameters of the physiological energetics of feeding:

(a) Food intake and feeding rates:

The difference between the quantity of food offered and the uningested
food yielded the food consumed per day. This value, when divided by the density of fish per tank yielded the food consumed per fish per day. The average daily food intake per fish was then expressed as mg dry food/fish/day. The feeding rate of the fish was calculated on the basis of the mid body weight (i.e. the mean of initial and final body weights) and expressed as mg dry food/g live fish/day.

(b) Food absorbed and absorption efficiency:

During the course of the 30 day experimental period, the respective media were changed once every 5 days. This water was then filtered through a previously weighed Whatman No. 1 filter paper to collect the faecal matter of the fishes. The faecal matter thus collected on the filter paper was air dried in the shade for 3 days. The filter paper with faecal matter was weighed regularly to obtain constant weight. The weight of faecal matter was then calculated as the difference between the initial weight of the filter paper and the final weight of the filter paper with the dried faecal matter. From this, food absorbed and absorption efficiency were calculated as follows:

\[
\text{Food absorbed (mg)} = \text{Food consumed (mg)} - \text{Faeces produced (mg)}
\]

\[
\text{Absorption efficiency (\%)} = \frac{\text{Food absorbed (mg)}}{\text{Food consumed (mg)}} \times 100
\]

The absorption rate was also calculated and expressed as mg dry food absorbed/g live fish/day.
(c) Growth and growth rates

The growth of the fish was determined using the sacrifice method of Maynard & Loosi (1962). Growth was obtained as the difference between the initial dry weight and the final dry weight of each group of fish, and expressed as mg gain in weight/live fish/day.

\[
\text{Growth rate} = \frac{\text{Gain in weight/live fish/day}}{\text{mid body weight}}
\]

The calculated rate is expressed as mg dry tissue/g live fish/day.

(d) Food conversion efficiencies:

The gross and net conversion efficiencies of the fishes are calculated as follows:

Gross conversion efficiency (\(K_1 \%\))

\[
= \frac{\text{Food converted (growth) (mg)}}{\text{Food consumed (mg)}} \times 100
\]

Net conversion efficiency (\(K_2 \%\))

\[
= \frac{\text{Food converted (growth) mg}}{\text{Food absorbed (mg)}} \times 100
\]

The calculated conversion rate is expressed as mg dry tissue converted/g live fish/day.
2.5 Biochemical analyses

After the completion of each feeding experiment, in each species of catfish, the biochemical analyses were performed.

The fish were dried in a hot air oven at 60°C and then the dried samples were used to estimate the water, ash, fat, carbohydrate and protein contents. These estimations were also carried out in control fishes prior to the commencement of each feeding experiment.

Water: Water content was obtained by calculating the difference between the live and dry weights of the fish.

Ash: This was determined by incinerating a known amount of the dried material (35 - 45 mg) in a Muffle furnace at 560°C for 5 hr (Paine, 1964).

Fat: It was determined by extracting a known amount of the dried material (35 - 45 mg) in a 2 : 1 chloroform : methanol mixture using a Soxhlet apparatus. As suggested by the Association of Official Agricultural Chemists (1965), each extraction was run for 20 hr.

Carbohydrate: This was determined by estimating the glycogen content by the phenol-sulphuric acid method of Dubois et al. (1956).

Organic nitrogen: This was estimated by the micro-kjeldahl method of Bradstreet (1965). A known weight of the sample was digested with concentrated sulphuric acid. The digested mixture was then distilled. The protein content was obtained by multiplying the organic nitrogen value by the factor 6.25.

While the contents of water and dry matter are expressed as percentages of the live weights of the material, all other biochemical constituents are expressed as percentages of dry matter.
Active individuals of *H. fossils* and *M. vittatus* were selected from the laboratory stock and used for the present study. The initial feeding was carried out in circular glass aquaria (diameter: 21 cm, height: 11 cm). Since pilot experiments conducted on *H. fossils* maintained at a density of 1 fish/aquarium, indicated a retardation of feeding, in the present series, the fish were kept at a density of 2 fish/aquarium, in 6 l of the respective medium. On the other hand, *M. vittatus* were placed individually in glass aquaria containing 3 l of the respective medium. Prior to the commencement of the experiment, all the fishes were starved for 3 days in order to ensure complete evacuation of the alimentary canal, and also to elicit hunger in these fishes (see Windell, 1967). On the 4th day, the fishes were fed with a known quantity of fresh muscles of the cyprinid *Rasbora daniconius*. The fishes were allowed to feed for 1 hr, after which the uningested food was removed, dried on a filter paper and weighed. Thus the live food intake of the fishes in 1 hr was determined, and expressed as mg food accepted/fish/hr. Following the serial slaughter method of Windell (1966), representative experimental individuals were sacrificed at intervals of every 15 min initially for a period of 1 hr, and subsequently at intervals of 1 hr, till the stomach of the fish was completely cleared of food. The alimentary canals of the fishes were dissected and opened to detect the presence or absence of food. The condition of the food and the pH of the stomach wall were noted by using the minute range pH comparator papers. The duration from the time of feeding till the stomach was completely evacuated of the food was taken as the total digestion period. The digestion rate of the fish was expressed as mg food digested/g live fish/hr.
2.7 Behaviour:

Behaviour experiments were conducted in a specially designed glass aquarium (40 cm x 25 cm x 40 cm), divided into 4 equal quarters using opaque plastic sheets, placed at right angles to each other. The aquarium was filled with 12 l of water (with or without the respective toxicant). The height of the water column was 16 cm.

Healthy individuals of the two species of fishes were used for observations on their 'surfacing activity'. The fishes were starved for 3 days in toxicant-free water and on the fourth day, one fish was released into each quarter and held for a total period of 2 days. Each day, at 10.00** and at 15.00**, the fish in each quarter was observed continuously for 30 min, to record the 'surfacing' as well as the opercular activities. The swimming behaviour was also observed. Each experiment was repeated with 2 sets of fishes (with 4 fish in each set), thus providing a total of 16 observations. The opercular activity was measured as number of opercular beats/min, while the frequency of 'surfacing' was calculated as the number of visits by fish to the surface of water/30 min.