III Materials & Methods

A. Sampling of Milkfish & Nile Tilapia at Commercial Setups

1. Monthly Changes in Phytoplankton Biomass & Composition

In order to be able to compare the food intake and composition of the cultured fish with the food availability at different times of the year, it was necessary to monitor the algal biomass throughout the study period. As mentioned previously, however (cf. Introduction), this study was part of a larger project to set up a management plan for Laguna de Bay, carried out by several institutions. The task of monitoring the limnology and water quality of the lake was assigned to SEAFDEC AQD and presented in the communal annual report to the funding agency, the European Union (SEAFDEC 1996, 1997, 1998). The work discussed in this section is essential to this dissertation insofar that it gives information on the changing availability of natural food for milkfish and tilapia and has been included as original work, since it was collected by an institution directly involved in the overall project.

Monthly water samples were collected at four stations in the lake (Station W: West Bay; Station C: Central Bay; Station S: South Bay; Station P: Fishpen; Fig. 3) from April-December 1995. After a project re-evaluation, Station C was dropped in 1996 and 1997. Each station was sampled once a month with the sampling time falling around midday on each occasion. Stations W&P were sampled one day and Station S (and C in 1995) the next day. On each occasion, integrated water samples (one per station in 1995, three in 1996-1997) were taken with a 3*l* modified Schindler type water sampler at 0.5m depth intervals throughout the water column with two samplers collected at each depth. The entire sample was pooled in a PVC container, a 1*l* subsample taken for the estimation of algal biomass and the remainder filtered for zooplankton analysis (not relevant here). The phytoplankton sample was preserved in Lugol's solution and analysed at a later date. For this purpose, a 10m*l* aliquot was concentrated to 1m*l* by centrifugation and resuspension and the phytoplankton identified and counted on a haemacytometer. The wet biomass was estimated by shape approximation assuming a relative density of 1.0 for all plankton groups. Each phytoplankton sample was analysed in triplicate and the results averaged.

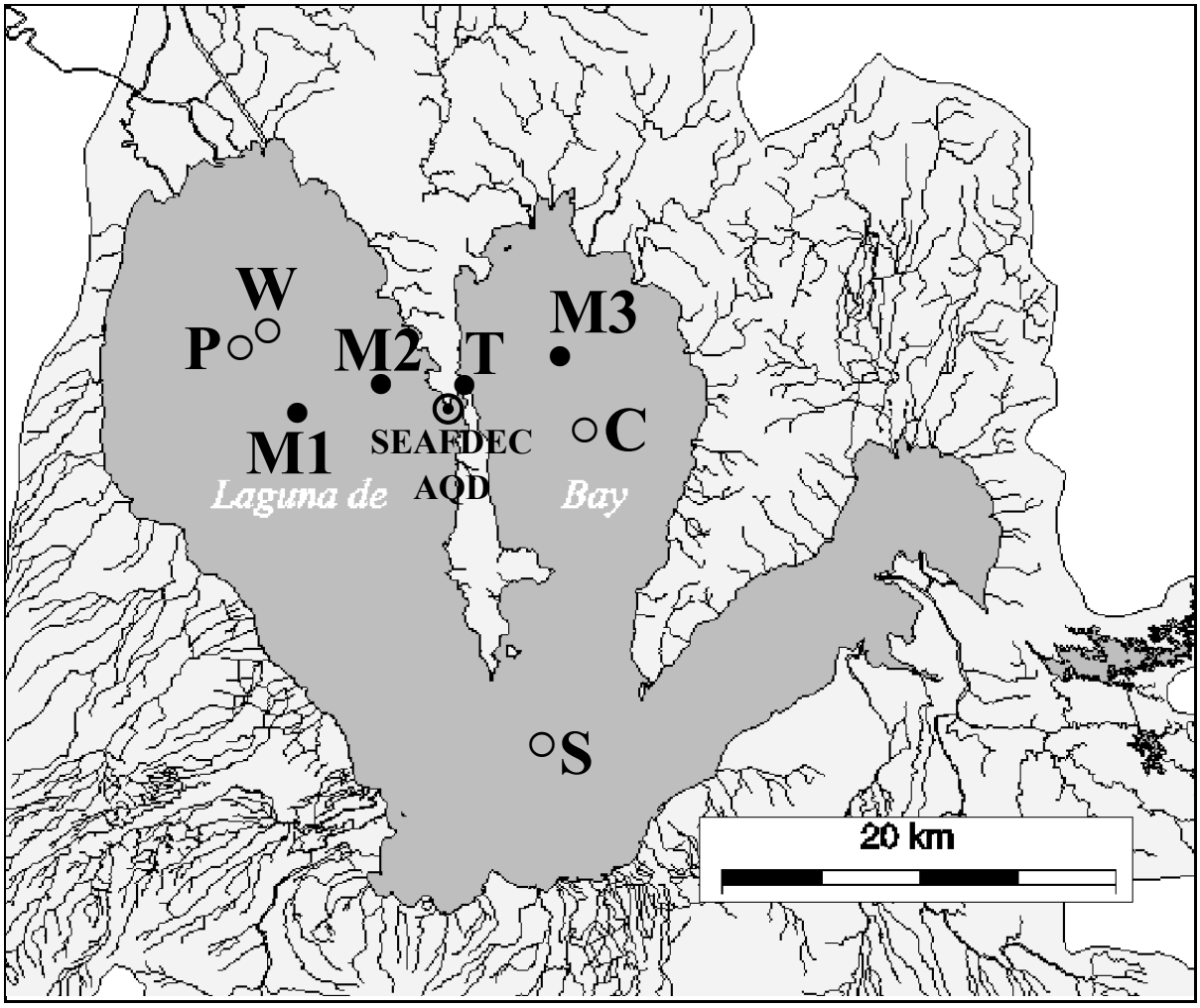

Figure 3. Map of Laguna de Bay and its watershed, showing the SEAFDEC water quality monitoring stations (○) and the location of the fishpens and -cages (●) at which fish sampling was carried out. SEAFDEC Stations: W (West Bay), P (Fishpen), C (Central Bay) and S (South Bay). Fish sampling sites: T: fishcages used for all tilapia samplings; M1: fishpen used for June and August 1995 milkfish samplings; M2: fishpen used for October 1996 and February and April 1997 milkfish samplings; M3: fishpen used for June and August 1997 samplings. SEAFDEC AQD, at which laboratory work was carried out, is included for reference (⊙). Diagram by courtesy of the University of Hamburg

2. Milkfish

a) General Sampling Procedure

Milkfish were sampled on several occasions throughout the study period with the primary intention of assessing their food composition and daily ration. Length and weight data were also taken on these occasions to estimate growth rates and condition and the gutted carcasses were also used for the analysis of body composition. In accordance with the

general requirements for estimating daily ration by stomach content modelling, the subsamples were spread more or less evenly over the 24-hour cycle. In 1995, eight subsamples of ten fish were collected at three-hour intervals but subsequent analysis demonstrated that the data gained from this sampling regime failed to meet the lower limit required for a reliable daily ration estimate. In the 1996-97 samplings, subsamples were therefore spaced at hourly intervals as far as the sampling circumstances allowed and the number of fish per subsample reduced to five.

Fish were obtained from a commercial operator on all sampling occasions so that they were cultured according to the practices commonly used in Laguna de Bay. In the case of milkfish, it was not possible to cooperate with the same fishpen owner over the entire study period. The first (sampled in June and August 1995) was put out of business by a severe typhoon towards the end of 1995 and the second (sampled October 1996, February and April 1997) increasingly came to regard the sampling days as disruptive until he refused to cooperate further so that a third fishpen had to be visited (sampled in June and August 1997). As a result, some differences with respect to fishpen size (100, 25 and 60 hectares respectively) and location (Fig. 3) had to be taken into account, but in all three cases, the fish were stocked at the same rate (ca. 5 fish m^{-2}) and kept without supplemental feed.

The general analytical procedure is summarised in Fig. 4. Due to the size of the fishpen, the only acceptable method of catching milkfish was by gillnetting. Milkfish in a particular pen are stocked on the same day and no fish are added or removed until they are harvested. At any one time, all the fish in the pen would therefore be expected to be approximately the same size. The mesh size of the gillnet was thus chosen accordingly after interviewing the fishpen operators as to what size of fish could be expected. After sampling, the fish were immediately killed and processed in the field. Standard lengths (nearest mm) and total and gutted weights (nearest g) were measured, the inner organs (liver, kidney, intestinal tract, gall bladder) were removed and placed in preweighed containers, after which gutted fish and innards were kept on ice until the return to the laboratory. Here, the fish were frozen until subjected to proximate analysis and the innards weighed (nearest 0.01g) and preserved in 70% ethyl alcohol until the stomach content analysis was carried out.

b) Growth Rates

The fact that milkfish in Laguna de Bay are stocked in empty fishpens and no fish are added or removed until harvesting makes it possible to estimate the growth rates of this

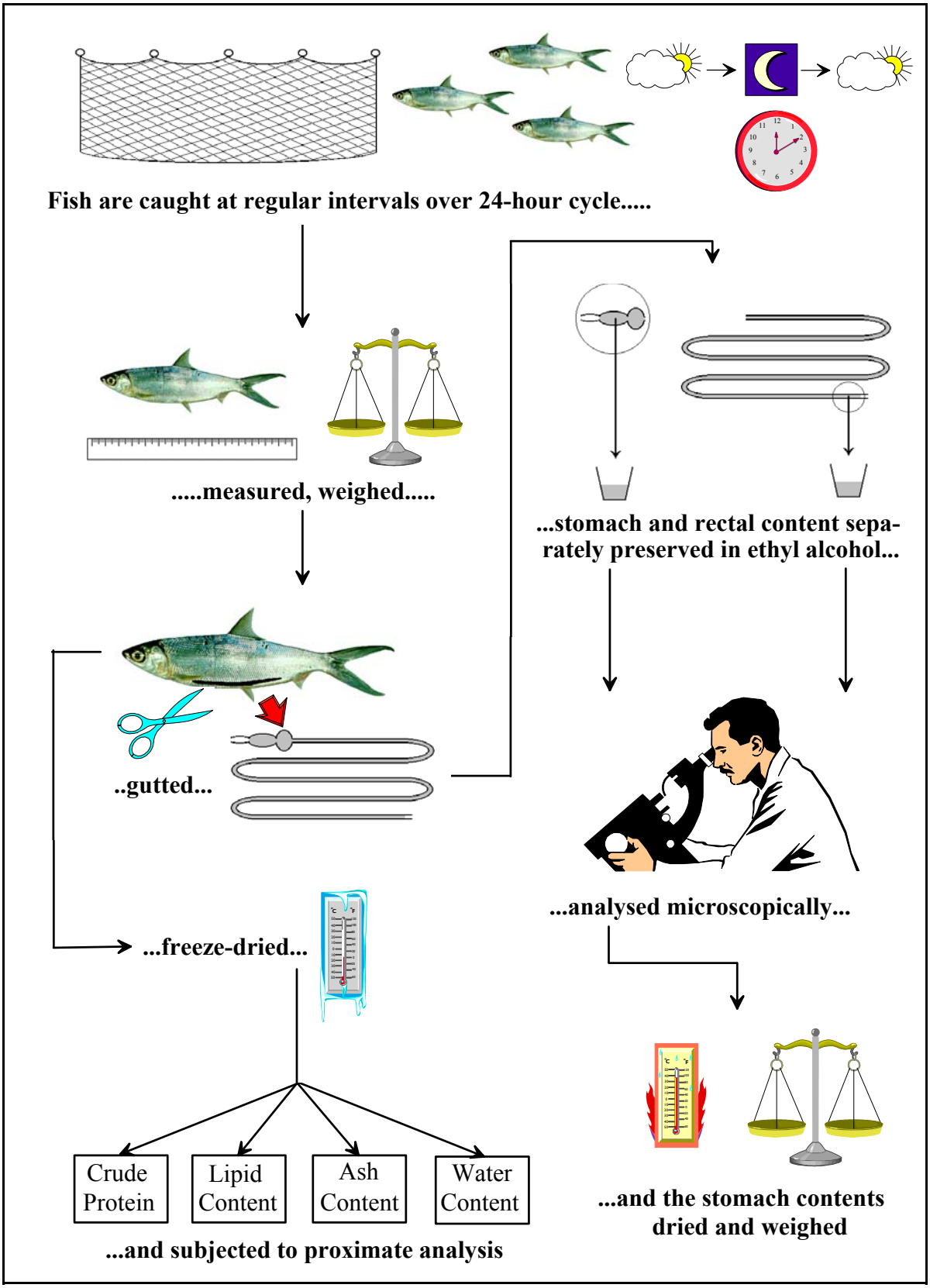

Figure 4. Schematic representation of the procedure by which milkfish and Nile tilapia and their stomach contents were analysed.

species for different times of the year provided that the same fishpen is visited twice and no harvesting has taken place in the time interval between sampling. The population Specific Growth Rates (SGR; %) and Metabolic Growth Rates (MGR; g $kg^{-0.8}$ day^{-1}) were therefore calculated for milkfish for those phases of the study period for which these conditions could be satisfied. The following formulae were used:

$$\mathrm{SGR} = 100 \times (\ln[W_b] - \ln[W_a])/t \qquad (29)$$

and

$$\mathrm{MGR} = ((W_b - W_a)/[((W_a/1000)^{0.8} + (W_b/1000)^{0.8})/2])/t \qquad (30)$$

(W_a = Average Total Fresh Body Weight at First Sampling (g); W_b = Average Total Fresh Body Weight at Second Sampling (g); t = Number of Days between First and Second Sampling)

c) Condition

The condition of milkfish was assessed by calculating the condition factor for each individual fish using the formula of Ricker (1975):

$$K' = 100 \times GW/SL^b \qquad (31)$$

(GW = Gutted body weight, fresh (g); SL = Standard length (cm); b = Coefficient of the regression between log[SL] and log[GW] for all fish from all sampling days)

Since milkfish were found to show pronounced diel feeding periodicity (cf. Results), the condition factors were based on gutted weights in order to avoid having this parameter influenced by differences in food consumption related factors, such as feeding intensity and length of the feeding period, between sampling days.

d) Body Composition

A sample of 24 of the gutted fish (one from each hourly subsample) was later homogenized and analysed for moisture, crude protein, total lipid, and ash content. Water content was assessed by loss of weight after freeze-drying and heating to constant weight at 105°C to remove residual water. Crude protein was estimated as total Kjeldahl nitrogen x 6.25, total lipid as the ether-soluble extract and subsamples were combusted at 500°C to determine ash content.

e) Food Composition

The preserved stomachs were opened by a longitudinal incision, the contents flushed out into preweighed containers and preserved in 70% ethyl alcohol until microscopic analysis. For this purpose, the stomach contents were fully mixed and a small subsample, sufficient to comfortably fit under a cover slip without air bubbles being trapped or excess sample to ooze out, transferred to a microscope slide. The entire subsample thus obtained was analysed, all taxa identified as far as possible and the percentage contribution of the major components (phyto- and zooplankton, benthic algae, benthic crustaceans) estimated visually according to their level of coverage. Components found only at trace levels were assigned a nominal 1%. The relative simplicity of the method and the homogeneity of the samples ensured that the analysis of one such subsample was sufficient; no discernible differences were found between replicates analysed for the earlier samples and the analysis quickly showed that the assessment of even a portion of the subsample under the cover slip would have sufficed. All unidentifiable material was attributed to detritus. This was present in abundance (cf. Results) but almost always as amorphous detritus (Bowen 1987). A rectal sample was also collected from the last few cm of the intestinal tract in order to be able to judge the state of digestion of those components observed in the stomach contents. After analysis under the microscope, the material on the slide was recombined with the remainder of the stomach contents of that fish. These were then dried at 70°C in the preweighed containers and the dry weights measured (nearest 0.01g) for the MAXIMS analysis.

f) Feeding Periodicity & Daily Ration

In order to compensate for the fact that larger fish are logically able to consume more and that the fish collected on any one sampling day were not of the same size, the dry weights of the stomach contents were standardised for the purpose of the MAXIMS analysis by converting them to percentages of the fresh weight of the fish (% Body Mass Equivalent, hereafter referred to as % BME).

The MAXIMS curves for any particular sampling day were calculated using the model in the version programmed for SAS® 6.11 for Windows (Richter *et al*. 1999). Since milkfish, as filter feeders, are more likely to have relatively constant ingestion rates, the MAXIMS Model 1.1 was used in all cases for this species. The SAS® routines used to model the milkfish samplings are given in Appendix 1. In all cases, the data points gained from the individual fish rather than the averages for the subsamples were used. Several different sets

of starting estimates were used and the fit with the lowest Sum of Squared Residuals (SSR) value selected. Using the SAS® output, the daily rations were calculated according to Eqn. 23 from the integrals of the feeding rate over the feeding phase.

The standard errors to the parameter estimates were taken directly from the SAS® output. The standard error to the daily ration was calculated by the method described in Richter *et al*. (1999) using the parameter estimates, their standard errors and their correlation coefficients (all given by the SAS® output). This involved the multiplication of the partial derivatives matrix with the covariance matrix and multiplying the resulting product with the inverse of the partial derivatives matrix, as described by Rasch (1976). An example of this procedure is given in Appendix 3. The equivalent parameter and daily ration estimates obtained for different sampling months were compared statistically using the Tukey-Kramer test for unplanned comparisons (Sokal & Rohlf 1995) at a significance level of $p \leqslant 0.05$.

3. Nile Tilapia

a) General Sampling Procedure

The analysis of tilapia proceeded on rather similar lines to that of milkfish, the main difference being that, since the commercial farm from which Nile tilapia were obtained was located inshore and close to the Binangonan Freshwater Station of the Aquaculture Department, Southeast Asian Fisheries Development Centre (SEAFDEC AQD; Fig. 3), it was possible to analyse the fish in the laboratory of this station and slightly simplify the procedure. The tilapia were kept in shallow water in small, closed-bottomed cages which measured about 3x6m and which were sufficiently deep to reach the bottom sediments. The stocking rates were approximately 50 fish m^{-2}, which is not excessive considering that this species is supplemented for large parts of the year; fingerlings are stocked at up to 200 m^{-2} (Guerrero et al 1987). Sampling was carried out in May and August 1995, March, May, July and September 1996 and January 1997. In May 1995, the fishcage contained two groups of fish of distinctly different sizes and both size classes were analysed separately. On each sampling occasion, fish were collected by manually lifting the net until a small enclosure was formed and randomly extracting the desired number of fish. These were immediately killed by immersion in iced water and brought to the station for analysis. Standard lengths (nearest mm) and total and gutted weights (nearest 0.01g) were measured, the inner organs (intestinal

tract, liver, kidney, gall bladder, gonads) removed, weighed (nearest 0.01g) and preserved in 70% ethyl alcohol. The fish were frozen until further analysis for body composition.

Supplemental feed (Robina Starfeeds, Universal Robina Corporation) was given in August 1995, September 1996 and January 1997; on the latter two sampling occasions, two sets of fish from different fishcages were analysed, one with and the other without supplementation in order to compare the two. In August 1995, the fish were offered pellets in one dose in the morning whereas in September 1996 and January 1997, powdered feed was given (one dose in 1996, two in 1997). This change reflected the economic situation of the operator: in November 1995, a strong typhoon destroyed practically all aquaculture structures in the lake so that even a year later, the farmer could not afford to buy pelleted feed but had to rely on stocks which were made up only of starter crumble. The level of supplementation was left to the discretion of the fishfarmer and therefore varied between sampling days. The quantity of feed commonly given to tilapia in Laguna de Bay ranges between around 6-12% Body Mass Equivalent (% BME) and partly depends on the financial situation of the fishcage operator. In our case, subsequent discussion revealed that the fish were given 6.5% BME in August 1995 and 8% BME in September 1996 (wet:wet ratio) in one dose in the morning. Due to a calculating error by the fishfarmer, the supplementation level was disproportionately high in January and the fish received nearly 40% BME in two doses, so that the fish can be regarded to have been fed to excess on that occasion.

b) Growth rates

These growth rates of this species were not calculated using the data from the fish sampled at commercial operations since on account of the culture method, it is easy and therefore apparently common practice in Laguna de Bay to check these fish and remove those that have reached a marketable size and/or add new fish. This would have distorted any population growth rates determined. In addition, the fact that these fish were given supplemental feed for at least part of the study period would have made it difficult to interpret the growth rates in relation to water quality and natural food availability. The growth rates were instead determined by keeping fish in cages at the SEAFDEC station over eight months in 1997 without supplementation (cf. Section B, Tilapia Growth & Water Quality Study).

c) Condition

The body weight range of the tilapia collected in this part of the study was rather less than two orders of magnitude so that any allometry in the growth of this species would not be expected to have much of an effect in the determination of condition of the fish sampled here. When assessing the condition of this species, Fulton's (1911) condition factor was therefore used:

$$K = 100 \times GW/SL^3 \qquad (32)$$

(GW = Gutted body weight, fresh [g]; SL = Standard length [cm])

The data for May 1995 (large and small fish) were combined since these had been kept under the same conditions, whereas the data for supplemented and unsupplemented fish collected in September 1996 and January 1997 were analysed separately. This was because fish kept with and without compound feed had been on these feeding regimes for about a month prior to sampling in order to allow them to acclimatize to the presence/absence of feed in their diet well in advance. As in the case of milkfish, distinct feeding and non-feeding periods were found over the 24-hour cycle (cf. Results) so that the gutted weights were used in the analysis of condition.

d) Body Composition

The proximate analysis of tilapia proceeded on the same lines as that for milkfish. The supplemental feed provided in August 1995, September 1996 and January 1997 was analysed in the same manner, although for this substance, total fibre was also determined.

e) Food Composition

The stomach contents of tilapia were also analysed in a similar manner to those for milkfish. When supplemental feed was given, this was distinguished from detritus on the basis of the amorphous nature of the latter. The fishfeeds used in the Philippines contain substantial proportions of plant material which retain their cell structure even when ground and there was no mistaking the characteristic, fine-grained detritus for supplemental feed.

f) Feeding Periodicity & Daily Ration

The dry stomach content weights of tilapia were transformed to % BMEs and analysed with the aid of the MAXIMS model (SAS® 6.11 Version) in order to investigate

feeding periodicity and food uptake. One problem in the case of this species was that only the fresh weights of the stomach contents were recorded for the 1995 samples, making a comparison with the data from subsequent samplings difficult. In addition to the dry weights, the alcohol-preserved weight of the stomach contents as well as the fresh weight of the digestive tract were taken for each fish in 1996 and 1997. This permitted an estimation of the fresh weight of the contents for the 1996 and 1997 by the following formula (adapted from Kühlmann 1998, his Eqns. 9&10):

$$FrWC=(FrWI \times AlcWC)/AlcWI \qquad (33)$$

(*FrWC* = Fresh Weight of Contents; *FrWI* = Fresh Weight of Full Innards; *AlcWC* = Alcohol preserved Weight of Contents; *AlcWI* = Alcohol preserved Weight of Full Innards)

These estimated fresh weights were then regressed against the respective dry weight for that fish by means of a Model II (geometric mean) regression (Sokal & Rohlf 1995). The regression coefficients for each sampling day were then averaged and used to convert the results of the 1995 samplings to estimated dry weights.

The main problem in applying the MAXIMS model to the data was that the basic assumption of a constant ingestion rate in the feeding period was seriously violated in several of the data sets collected. This mainly happened when supplemental feed was given since this component was ingested far more easily than natural food, but other reasons for this were also found (cf. Results). Irrespective of the causes of this phenomenon, none of the normal MAXIMS routines could be applied and another solution had to be found.

By analysing not only the total weight of the stomach contents over time but also their composition, it was possible to determine when one food component (e.g. supplemental feed) started to increase or decline relative to the other components. These time points were taken to mark a change in the feeding pattern and the feeding period was split into several subphases on the basis of this information. Each subphase was assumed to have a constant ingestion rate (equivalent to the MAXIMS Models 1.1 & 2.1 assumption) but different subphases were allowed to differ with respect to this parameter. The instantaneous evacuation rate was assumed to be constant throughout the 24-hour cycle. The entire analytical period was then remodelled by writing more complex models on the basis of the above assumptions.

All special models incorporated the same assumption concerning stomach evacuation common to all MAXIMS models, namely that stomach evacuation takes place at all times

and is directly dependent on the level of stomach fullness in combination with a constant instantaneous evacuation rate. A second common assumption was that the analytical cycle lasted 24 hours and that the level of stomach fullness at the start was equal to that 24 hours later. One vital prerequisite was that the fish ceased feeding for at least part of the study period so that the model was able to calculate the instantaneous evacuation rate. The SAS® routines written for tilapia are presented in Appendix 2; further details of the individual models are presented in Section III: Results. Again, the individual data points were used rather than the subsample averages and the best fit was chosen on the basis of the lowest SSR value. The daily rations were again calculated by integrating the ingestion rate(s) over the (respective) feeding phase(s) analogous to Eqn. 23 & 24. The confidence limits to the daily ration and the comparison of parameter and daily ration estimates were calculated according to the same principles as for milkfish.

B. Tilapia Growth & Water Quality Study

1. Water Quality Sampling

a) General Sampling Procedure & Secchi Depth

Between March and November 1997, water samples were collected at weekly intervals for particulate organic and inorganic matter (POM, PIOM), Chlorophyll-a (Chl-a) and zooplankton biomass at the SEAFDEC station. The Secchi depth was also measured on these occasions. Integrated water samples were taken with the aid of a 3*l* water sampler (modified Schindler type) at depth intervals of 0.5m starting at the surface; two full samplers were collected at each depth interval. All water thus obtained was pooled in a large PVC container and filtered first through a 50µm, then a 15µm plankton net. The residues were resuspended in 300m*l* distilled water and a quantity of the filtrate sufficient for further analysis retained so that three size fractions (<15µm, 15-50µm and >50µm; hereafter referred to as the small, middle and large size fraction respectively) were collected. This procedure was repeated twice so that triplicate samples were collected for all size fractions, which were kept refrigerated until further analysis on the same day as the samples were taken.

b) Chlorophyll-a

100m*l* of each sample (replicate and size fraction) was filtered onto cellulose nitrate filters and crushed in 10m*l* acetone (90% concentration) to extract the photosynthetic pigments. The sample was centrifuged and a spectrophotometric reading taken at 665nm. The Chl-a concentration in the water was calculated from the following formula, adapted from Golterman *et al*. (1978):

$$Chl-a = SR \times 11.9 \times v/V \qquad (33)$$

(*Chl-a* = chlorophyll-a concentration [mg l^{-1}]; *SR* = spectrophotometric reading at 665nm; *v* = volume of acetone used for extraction [m*l*]; *V* = volume of water sample filtered [m*l*])

When conducting this calculation for the middle and large size fractions, the degree to which these had been concentrated by the filtering process was taken into account.

c) Particulate Organic & Inorganic Matter

A further 100m*l* of each sample (replicate and size fraction) was filtered through preweighed Whatman GF/C glassfibre filter papers to assess the concentration of suspended solids. The filters were dried at 70°C after filtration, weighed (nearest mg), ashed at 550°C and weighed once more (nearest mg). The difference between weight after ashing and initial filter weight was taken as the level of PIOM; the difference between weight after drying and weight after ashing as the level of POM.

d) Zooplankton

A further three replicate water samples were collected for zooplankton analysis. These were filtered through a 50µm plankton net; it was assumed that all zooplankton was large enough to be retained by this mesh size. While this was not strictly true for some of the smaller rotifers, it is certainly true that all the copepod and cladoceran species would not pass through this pore size so that most of the zooplankton may be assumed to have been included in the sample. The residues were resuspended in 10% methanaldehyde (formalin) solution to preserve them until further analysis. At a later date, a representative portion was analysed under the microscope, the zooplankton counted and its biomass estimated by shape approximation assuming a relative density of 1.0 and a wet:dry mass ratio of 10:1 (Schwoerbel 1980).

2. Tilapia Growth Rates

Since it had not been possible to calculate the growth rates of Nile tilapia from the fish used for stomach content analysis on account of their culture method, growth rates for this species were obtained by keeping fish in cages in the lake. This was done at the SEAFDEC Station during the same time period as the water samples for Chl-a, zooplankton, POM and PIOM were collected. No supplemental feed was given and the standard length and total weight of the fish were determined twice a month. Four replicate cages, each measuring 5x5x4m, were used and the initial stocking density was 25 fish per cage. This figure is rather lower than the 50 fish m^{-2} normally stocked in the case of this species but it should be remembered that in this study no supplemental feed was given. The fish were collected for analysis at around 8:00 hours on all measuring/weighing days and kept in clear water so that substantial differences in weight and condition between different sampling days due to different levels of gut fullness were not to be expected. The fish were blotted dry and the water drained from the oral cavity to minimise sampling error; this practice also helped determine whether any of the fish were mouthbrooding and thereby prevented from feeding. The fish were not tagged so that it was not possible to monitor the growth of individuals; nevertheless, all fish were measured and weighed individually. Following analysis, the fish were returned to their cages. Dead fish were not replaced in order to simplify the calculation of population growth rates between samplings. Stocking took place on 26. March 1997 and the last analysis was carried out on 20. November 1997.