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Use of Macroalgae to Solve Water Quality Problems in Intensively Cultured Marine Fishponds

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ABSTRACT

The performance and nutrients (N, P) transformations of seaweed (*Ulva lactuca*) biofilters under field conditions were studied. The algae grew well on fishpond effluents. The year-round growth averaged 200 g wet weight (30 g dry weight) $\text{m}^2 \text{ day}^{-1}$. $\text{NH}_3\text{-N}$ removal ranged from over 90% at $\text{NH}_3\text{-N}$ fluxes of $2 \text{ g m}^2 \text{ d}^{-1}$ to 60-70% at $8 \text{ g m}^2 \text{ d}^{-1}$. At higher ammonia fluxes, uptake rates of $7 \text{ g m}^2 \text{ d}^{-1}$ could be reached. Only a moderate seasonal effects were observed in water quality.

These data served in the design of a recirculating integrated system for the culture of fish and seaweed. The system contained a high biomass of fish (*Sparus aurata*), yet the seaweed biofilters removed most of the toxic metabolites from the water. A large fraction of the water was recycled, reducing pumping costs and retaining valuable nutrients as produced biomass. The system showed improved stability and control of the water quality compared to existing systems and was more reliable and robust to human error and natural events.

Several total nutrient budgets were measured. Seaweed uptake, ammonification, and nitrification were the major processes in the system, processing on average 17-39%, 16% and 10%, respectively of the N input. Seaweed uptake was the only major P-processor, at 9-21% of the total P-budget. Denitrification and sulfate reduction were two significant anaerobic processes (about 5% of the N-budget each).

The model system of 9000 l and 10 m^2 generated yields of fish and seaweed, averaging (over two years of operation) 0.14 and 1.15 kg d^{-1} , respectively. FCR was 4.3, and each kg of fish feed generated nearly 2 kg of seaweed.

A computer simulation model of the N-cycling in and integrated system was constructed with the data gathered. The model reproduced closely the experimental results, at an extremely good level for a process-based ecological model. This implies that the model correctly includes the key processes operating in this system. The model is available for aiding the design of full-scale culture systems of this type.

COMMENTS OF THE PI ON THE RESULTS OF THE PROJECT IN VIEW OF THE ORIGINAL OBJECTIVES

All the objectives of the original proposal, as reproduced below, have been achieved, as is clear already from the abstract above. The technology of integrated fish and seaweed culture has been proven to fulfill all its promises. Several studies and projects in the NCM have already used the concept (see for instance the enclosed reprint of Shpigel et al., 1993). A modification, for the integrated culture of abalone (*Haliotis* spp.) and *U. lactuca* is already under field trials at the NCM. Some of the information has also been presented in the US, for example to the demonstration mariculture farm project in Cape May, New Jersey. We thus feel that our project has been very useful to mariculture in Israel and the US.

Six publications and one M.Sc. thesis have already been produced in association with this research (and the chapters in this report will form at least three more publications):

1. Cohen, I., Neori, A., 1991. *Ulva lactuca* biofilters for marine fishpond effluent. I. Ammonia uptake kinetics and nitrogen content. Bot. Mar. 34:475-482
2. Neori, A., Cohen, I., Gordin, H., 1991. *Ulva lactuca* biofilters for marine fishpond effluent. II. Growth rate, yield and C:N ratio. Bot. Mar. 34:483-489
3. Neori, A., 1991. Use of seaweed biofilters to increase mariculture intensification and upgrade its effluents". Fisheries and Fishbreeding in Israel: Review of Fisheries in Israel 24(4):171-179 (In Hebrew, with an English Abstract).
4. Shpigel M., A. Neori, D.M. Popper and H. Gordin, 1993. A proposed model for "environmentally clean" land-based culture of fish, bivalves and seaweeds. Aquaculture 117:115-128.
5. Neori, A., S.P. Ellner, C.E. Boyd and M.D. Krom, 1993. The integration of seaweed biofilters with intensive fish ponds to improve water quality and recapture nutrients. In Moshiri, G.A. (ed), Constructed Wetlands for Water Quality Improvement, Lewis Publishers, Boca Raton, Florida, pp. 603-607.
6. Tsai, K., S. P. Ellner and A. Neori, Submitted. A modified Steele's equation for the effect of irradiance on growth rate in marine phytoplankton. Submitted to Limnol. Oceanogr.
7. Davison, P. J., 1992. A Comparative Study into the Efficiency of an *Ulva lactuca* Biofilter, Eilat, Israel. An M.Sc dissertation presented to the University of Leeds, UK.

OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

I. Macroalgal biofilters will remove excess dissolved nutrients and stabilize water quality in intensive fishponds:

It is proposed to study the use of macroalgal (*Ulva* sp.) biofilters to remove metabolites from recirculated water of intensive fish (*S. aurata*) ponds. This treatment will dampen the water quality fluctuations, a characteristic which interferes with the present pond operation. The size of the biofilter, the macroalgal biomass in it and the rate of water recirculation between it and the pond will be controlled. The proportions of these variables which keep low levels of ammonia and of phytoplankton in the fishpond continuously will be determined. The resulting system will be more reliable, easier to control and more robust to human error or natural events.

II. A mathematical model will synthesize the available data into a useful research and operation instrument.

The dynamics of the interactions between the phytoplankton and the *Ulva* growth, the nutrients concentration, and the operational parameters of the system, will be studied with the aid of a detailed mathematical model. This model will combine the existing data, from earlier models and from previous experiments. It will be improved and refined successively, using new information gained during the proposed experiments. The use of total nutrient budgets will provide the quantitative chemical data on the dynamic interactions of the processes which control water quality in the system. The integration of this information to the model will enable the identification of the factors and processes of importance in the fishpond-algal interactions. Such a high level of perception is essential to the optimization of the integrated system, and probably also to its daily operation. By the end of the project we expect to have produced a solid and easy to use simulation model. It will operate on a PC computer, making it a handy aid to the fishpond manager in the day to day operation of the pond-macroalgae system.

FINAL REPORT

Chapter I: A NOVEL DESIGN FOR INTENSIVE FISH-CULTURE INTEGRATED WITH SEAWEED BIO-FILTRATION PROVIDES LONG-TERM STABLE WATER QUALITY

I. INTRODUCTION

Intensive mariculture, the farming of marine organisms, is a relatively recent development with great commercial potential. Until now much of the attention world wide has been given to cage mariculture, with extensive commercial developments in Japan, Scotland, Scandinavia and the American North-West amongst other places. However, the limited availability of suitable sites and pollution problems caused by the waste materials from the cages have been causing problems for the industry (Gowen et al., 1989, Watanabe, 1991). The major alternative to sea-cage mariculture is the growth of fish in coastal seawater fishponds. As with freshwater ponds, water quality limits the biomass which can be grown in seawater fishponds and hence their profitability (Rimon and Shilo, 1982). A secondary, but non the less important problem, is pollution caused by the effluents downstream from the culture facility (Boyd, 1990; Bergheim et al., 1991; Cripps, 1991).

Preliminary results in the intensive seawater fishponds in Eilat (Neori et al, 1989) have shown that the major water quality problems which can arise at various times in these ponds are low dissolved oxygen at night, high dissolved oxygen during the day, high dissolved ammonia and high or low pH. These water quality problems alone or combined with each another, often followed by various pathogens, represent the major threats to the well being of the organisms being cultured in the fishponds.

Once dissolved oxygen levels are controlled by aeration, the next most important water quality problem in fishponds is the accumulation of ammonia in the water column (Boyd, 1990). It was initially thought that it would be possible to use phytoplankton to remove dissolved ammonia. So far, the studies on these ponds have shown that this effective solution is not dependable enough. For most of the time the ammonia-N is indeed taken up rapidly by phytoplankton (Neori et al, 1989; Krom and Neori, 1989) and the water chemistry is thus maintained within acceptable limits. However, there are periodic crashes of the phytoplankton, caused by micro-flagellate grazing (Neori et al., 1989 and unpublished) which result in short periods when there are high levels of ammonia and low levels of oxygen in the water column. This combination of conditions has resulted in mass mortalities of fish in the semi-intensive ponds in Eilat (Krom et al 1985a). As with most other fish, *Sparus aurata*, the principal cultured organism in Eilat, assimilates only 20-30% of the food it is fed for growth (Krom et al 1985b, Porter et al 1987; Krom and Neori, 1989; Krom et al., in preparation; Lupatch, unpublished). The remainder is excreted. This results in a high nutrient load which, if not removed, will pollute the pond and the water downstream.

Pollution problems of the kinds described here exist for all aquaculture facilities and are correctly receiving increasing attention. It is likely that any aquaculture facility which does not treat its effluent in some acceptable manner will simply not be allowed to operate (See several papers in Cowey and Cho, 1991). However, at present there is no established technology available for the removal of nutrients from polluted seawater. For instance, bacterial nitrification/denitrification biofilters, common in freshwater aquaculture, combine aerobic and anaerobic stages. The latter, in the presence of the high seawater sulphate levels (30 mM and above) can quickly produce hydrogen sulfide. Furthermore, such biofilters add to the production cost while bringing no income to the grower.

Ponds with macro-algae, or seaweed, provide an attractive alternative to phytoplankton ponds and bacterial biofilters for the control of water quality in fishponds and their effluents. Unlike phytoplankton, the

seaweed biomass and hence nutrient-uptake rate can be controlled over long periods of time, the system is aerobic and produces a useful biomass. A major advantage for the use of macro-algae to treat the effluent from the fishponds and other culture facilities is the production of high-quality macro-algae which will be commercially useful either directly or as food for a second organism.

The use of macrophytes has been tried in freshwater systems (Boyd, 1979; Athie and Cerri, 1987). Surprisingly little work has been carried out on seawater pollution control (Ferguson-Wood and Johannes, 1975). Frederiksen (1987) has described the reaping of natural *Ulva lactuca* beds as a way to remove excess nutrients from the inlet to a fjord. In France, the effects of *Ulva* production on water quality in polluted estuaries has been under investigation (Ph. Morand, pers. comm.).

Ulva sp. has been suggested to be particularly suitable for large-scale culture in polluted seawater (Ryther, 1983). The relative resistance of *Ulva* to high levels of various pollutants and nutrients makes this genus very useful in water pollution control. Laboratory experiments have shown macro-algae, including *Ulva*, to have very high uptake capacity for ammonia-N (Lobban et al., 1985). Typical uptake values range from 1.8-2.9 mmol m⁻² h⁻¹. The results from outdoor experiments in Eilat, on the uptake rate of *Ulva* after starvation have reached short-term values of up to 120 mmol m⁻² h⁻¹ (Cohen and Neori, 1991; Vandermeulen and Gordin, 1990). The values found under more normal and continuous conditions of growth are 10 mmol m⁻² h⁻¹ or 17 μ mol l⁻¹ h⁻¹. Our results so far (Cohen and Neori, 1991) show that at ammonia-N fluxes of up to 10 μ mol l⁻¹ h⁻¹ (0.14 mole or 2 g ammonia-N m⁻² d⁻¹) *Ulva* could strip out of fishpond effluents in one pass over 90% of the ammonia-N. At quadruple fluxes, ammonia-N stripping efficiency dropped to 60-70%, but the aerial removal rate reached 4 g ammonia-N m⁻² d⁻¹, and at increasingly higher fluxes, uptake rates of 7 g ammonia-N m⁻² d⁻¹ could be reached.

The performance of *Ulva* under field conditions has been studied, and we have developed the technology to a level in which we can keep *Ulva* tanks free of contamination by other algal species for extended periods. We have also found that the algae can grow at extremely high rates on merely fishpond effluents. The year-round growth rate achieved in 600 liter, 1 m² tanks, averaged 200 g wet weight (30 g dry weight) m⁻² day⁻¹ (Neori et al., 1991).

At the same time we studied the growth of young fish (*S. aurata*) in fishpond effluents after it was treated in *Ulva* tanks. The results have shown that the fish grew in the treated water even better than in the control of fresh seawater (Porter, Popper and Neori, unpublished). It is thus apparent that good results can be expected from an integrated system, in which water is recirculated between fishponds and seaweed ponds. Based on our data cited above, it was possible to make tentative calculations on the size of *Ulva* tanks required to treat any given ammonia production in such a fishpond.

The production of a kg of the seabream, at a food conversion ratio of 3 (dry pellets used per fresh fish produced) is accompanied by the release to the environment of approximately 110 g N (Krom and Neori, 1989). At an average ammonia-N removal rate of 3 g m⁻² d⁻¹ (Cohen and Neori, 1991), it would require approximately 37 m² days of a seaweed pond. In other words, for the complete removal of excreted N from the production of 1 t of fish, at a feeding rate of 1% body wt, or 10 kg feed d⁻¹, should require 120 m² of seaweed pond, which would produce up to 50 kg fresh seaweed (8 kg DW).

The present paper describes an integrated pond system containing a high biomass of fish (*S. aurata*) in which macro-algae remove most of the toxic metabolites from the water. A large fraction of the water was recycled, thus both reducing pumping costs and retaining valuable nutrients as produced biomass. The combination of conditions of fish biomass, water flow rate and macroalgal biomass was such that the levels of ammonia, dissolved oxygen and pH remained within safe limits continuously. The system showed improved

stability and control of the water quality compared to existing systems and was more reliable and robust to human error and natural events.

II. MATERIAL AND METHODS

A. System design criteria

The design of the experimental system was based on the data available at the time on water quality and the intensive culture of the gilthead seabream *Sparus aurata* (Neori et al., 1989; Krom and Neori, 1989; Krom et al., 1989; Gordin et al., 1990; Neori and Krom, 1991), and on the functioning of the green seaweed *Ulva lactuca* as a biofilter for fishpond effluents (Cohen and Neori, 1991; Neori et al., 1991; Neori, 1991). The recirculating fish-seaweed biofilter system was designed to maximize fish density in order to release as much culture space as possible for the seaweed biofilters. It was hoped that the design would allow most of the fish-excreted N to be converted to seaweed biomass. The rapid recirculation of water between the two compartments, the fish and the seaweed, was planned to allow a significant reduction in the use of clean seawater, a major cost item in a mariculture fish-farm. The experimental system consisted of a series of middle-size fiberglass tanks

Two systems were examined:

1. a model made of 4 identical 2.7 m³ trough tanks, a 400 l sedimentation tank and a 600 l polishing tank.
2. a pilot system, made of two identical 100 m² ponds. Each system operated for over two years almost continuously.

B. The model system

1. Design

The model system consisted of a series of fiberglass tanks (2.5 m³, 3.3 m² each) and two smaller tanks (Fig. 1). The design and operation were as follows:

- a. Nutrient load, and the resulting deterioration of water quality in a given intensive fishpond is determined by the fish feeding rate. The model system was going to be stocked with fish at densities which, according to their size, would consume daily a constant quantity of feed ration comparable to 4.5 kg d⁻¹, the ration used safely in the 100 m² ponds in the NCM (Gordin et al., 1990). For a total tank area of 13.3 m², This ration was 0.6 kg d⁻¹. With such a feeding rate we were confident that even if the biofilters fail, ammonia concentrations would not reach lethal levels (Neori et al., 1989; Wajsbrot et al., 1991).
- b. The fish required to stock the four-tank 10 m² system were concentrated in only one of these tanks (FT), and the remaining three were designated as seaweed biofilter tanks (UT1, UT2 and UT3), which were connected to the FT outflow in parallel (Fig. 1).

Figure 1. A schematic configuration of the integrated system for the culture of fish and seaweed. Tanks: A, fish-tank (FT); B-D, seaweed tanks (UT1, UT2, UT3, respectively); E, polishing tank (D); F, sedimentation tank (SD); G, recirculation pump. Major water flows: 1, inflow of clean seawater; 2, sludge drainage of SD tank; 3, main outflow.

- c. The water from the FT were pumped to the three parallel UT via a 400 l sedimentation tank (SD). An area of 10 m² (three tanks) was allocated to the seaweed biofilters based on the documented maximal daily ammonia removal rates of 2.5 g m⁻² (Cohen and Neori, 1991). The daily food ration of 600 g was expected to produce up to 27 g of excreted combined N (Porter et al., 1985); Krom and Neori, 1989; Lupatch, unpublished), or a reasonable 2.7 g N m⁻² of biofilter d⁻¹.
- d. The seaweed tanks were aerated at a moderate rate by a perforated air pipe, which was imbedded in the round bottom during manufacture. The 2.5-3 mm holes were spaced at 25 cm intervals. The large bubbles produced were sufficient to vertically stir the seaweed at a slow pace, as required for efficient seaweed cultivation. The water from the SD entered the UT in one side. Screens (5 mm mesh) at the other end kept the seaweed from leaving the tanks with the water. The water flowed out of the three UT back into the FT by gravity via stand-pipes. Clean seawater (inflow) flowed into the FT (usually at a daily rate of 2000 l). A water volume equal to the inflow, less evaporation, leakage and sampling, overflowed into a 600 l seaweed culture tank of the design described in Vandermeulen (1989), the polishing or D (for Dolav, the manufacturer) tank, and from there drained to the sea outfalls of the NCM (Fig. 1).
- e. The rate of water recirculation between the FT and the UT was determined at 800-1000 L h⁻¹, so that each UT tank, which were filled to about 2170 L, would receive between three and four full daily water exchanges (Vandermeulen and Gordin, 1990). For the FT, which was filled to about 1700 L, this water flow was equal to 11-14 full daily water volumes exchanges, added to a little over one daily exchange by the inflow. Thus, the fish experienced daily 12-15 water exchanges, over 90% of which were of recirculated water.

2. Operation

The system was operated practically at an identical protocol from the spring of 1991 to the fall of 1993:

- a. The seaweed were stocked at a density of 3 kg per tank, were harvested weekly and restocked at the original level. The weekly yield was the harvested seaweed quantity in excess of 3 kg.
- b. The fish were given their full feed ration (600 g) daily at 0830, in a demand feeder. In winter, when temperature dropped below 15 centigrade, and the fish appetite was reduced, a 3 KW heater was placed in the FT and the tank walls were insulated, to increase the day temperature to the 17-19 degrees required as a minimum for good appetite by the gilthead seabream (Popper, unpublished).
- c. Each day, at 0830 and at 1630, the inflow valve was opened for 4 h at an hourly rate of 250 l.
- d. Each day at 0830, the SD tank was drained for a few seconds to remove accumulated particles. Once every few days this drained sediments were collected, dried and quantified.

C. *The pilot system*

1. There were only two compartments: a fishpond, of the type described in Neori et al. (1989), with an area of 100 m² and a volume of 100 m³, and an Ulva pond, which was converted from an identical fishpond with the same dimensions by the installation of aeration pipes at the bottom. Water was recirculated continuously between the two ponds at a daily rate of 400 m³. In addition, the fishpond received daily 100 m³ (4m³ h⁻¹) of clean seawater.
2. The system was compared with a neighboring fishpond operated at the conventional, phytoplankton-dependant, operation mode, with a daily water exchange rate of 50% (Neori, 1991; Neori et al., 1989).
3. While the seaweed pond and the conventional fishpond were exposed to full sunlight, the fishpond in the integrated pilot was covered with a net which cut out 85% of the light, to prevent phytoplankton from outcompeting the seaweed.
4. The pilot was operated at two levels of fish biomass, requiring different daily feeding rates, first (September-November, 1990) of 10 kg and then (December, 1990-April, 1991) much higher, up to 20 kg d⁻¹. At the same period the conventional fishpond was stocked with a fish biomass which received a constant daily feed ration of 6.5 kg.
5. Basic water quality parameters (DO, Temp. and NH₃ levels) were measured in the three ponds daily or thrice weekly.

D. *Water sampling*

Water samples were routinely taken daily or thrice weekly from the different compartments. The samples were passed via a 1 mm screen and split to two parts. One served for electrode analyses, which were made each time, and the other for autoanalyzer chemistry of nutrients when and as required. All methods and instruments were standard, as described earlier (Neori et al., 1989; Krom and Neori, 1989; Shpiguel et al., 1993). Occasionally water was sampled also for particulate chlorophyll concentration and for microscopic counts of bacteria (using the DAPI stain method) and larger microorganisms (using live samples immobilized by formalin fumes).

E. *Hydrological Budget*

To determine a nutrient budget accurately, it is essential to have an accurate estimate of the systems hydrological regime. An investigation was therefore initiated to determine the volume, flow rate and rate of evaporation which define this systems hydrology. The investigation was divided into three parts; Initially a volumetric determination, then salinity measurements to calculate evaporation rates, and finally an estimation of water lost to leaks. All measurements were conducted on a clean empty system filled to operating capacity with the circulatory system switched off.

Due to the large volumes of water involved (2000 l for each of the individual system components) volumetric determination was conducted by dilution. A solution of known concentration was introduced into the system tank of interest. The tank was then aerated for half hour before duplicate sample collection to ensure thorough mixing. The introduced solute concentration was then determined for each tank.

Tank Volume = Concentration in original solution * Volume of original solution / Concentration of solute in tank

Salinity changes were not measured in a circulating system as water flow rates between tanks could not be defined with sufficient accuracy to yield viable results.

Samples for salinity determination were collected from each of the system tanks every two hours from 11:00 until 17:00 and again from 08:00 until 12:00. Sample conductivity was measured with an inductively coupled salinometer and salinity calculated using the International Oceanographic tables (UNESCO and National Institute of Oceanography, Australia). Samples were calibrated against a vial of standard sea water of 35 ppt salinity and chlorinity of 19.3750/00 (Institute of Oceanographic Sciences, Wormley, Surrey). Calculations of evaporation were based on Krant et al (1982).

$$(1) V * dSo = (Fi * Si - (Fi - E) * So) / dt$$

Where V is the volume of the tank, So is outflow salinity, Si is inflow salinity, t is time, Fi is inflow volume and E is evaporation. In this case Fi = 0, so:

$$(2) V * dSo = -E * So / dt$$

Integrating and solving this equation becomes:

$$(3) \ln (St/So) = - (E * t) / V$$

Values for salinity over the 24hr period were plotted on a graph of $\ln (St/So)$ against t. Evaporation rate given by Slope * V. The salinities found in the system have a quasi-linear relationship with a small variation between diurnal and nocturnal evaporation. We assumed therefore that evaporation was not a fluctuating phenomenon, and so defined a single rate for the 24hrs.

Significant leaks from the system could be seen and so sealed (the system tanks are elevated), and therefore the major losses from the system occurred primarily as a product of cleaning procedure, and so were directly measured.

F. *Analytical Methods*

All methods were standard, as described earlier (Krom and Neori, 1989; Neori et al., 1989; Cohen and Neori, 1991; Neori et al., 1991). Analysis of total dissolved N and P (TDN; TDP) was performed by a modification of the persulphate oxidation procedures described by Solorzano and Sharp (1980) and by Valderrama (1981). We used titrations of each sample series to assure reaching the desired pH values of 12.6 and 2.6, optimal for total oxidation of N and P compounds, respectively (Solorzano and Sharp, 1980). The concentrations of the reagents used were NaOH, 1.5 M; $K_2S_2O_8$, 0.224 M; HCL, 3M. The procedure was as follows:

1. 30 ml sample
2. add 0.224 M potassium persulphate ($K_2S_2O_8$) solution in 1.5 M NaOH, until pH reaches 12.6
3. autoclave 30 min at 121°C, cool (sample safe for storage at room temperature)
4. reduce pH with 3 M HCL to pH 2.6

5. autoclave 30 min at 121°C
6. cool and analyze with autoanalyzer for phosphate and nitrate

Electrode measurements were taken routinely twice each week day (08:30 and 13:30). We consider such measurements diagnostic and not analytical. Temperature and DO were measured *in situ* with a YSI oxygen electrode which was calibrated with DO-saturated seawater before each sampling. Samples for pH and ammonia electrodes were taken to the laboratory in 250 ml capped bottles. Measurements were made within a few minutes of sampling. The pH electrode was calibrated daily. The ammonia electrode (Ingold NH₃ electrode type 15 230 3000) was calibrated once a week using the procedure proposed by the manufacturer.

III. RESULTS

A. THE MODEL SYSTEM

The data for the model system are presented in two sets, one covering the period from January 91 to June 92, and the second covering August 92 to May 93. The separation is for visual convenience. Also, the first set includes two daily measurements (08:30 and 13:30), while the second data set includes only one daily sample (08:30). There is an additional data set of regular autoanalyzer analyses of the nutrients from 92-93. The figures with these data span the entire period, as they include also data from experiments beginning in May 91. The reader's attention is also drawn to Table 1, containing pertinent information on events which occurred during the project and which might have influenced the results.

Daily water quality parameters (AM & PM, when available):

Most water quality parameters, except temperature, were maintained in the system rather uniform throughout the day and the year. Most of the more extreme values in the data resulted from mechanical "childhood problems" (a pump breakdown, a flooding of aeration pipes, a broken standpipe etc.). The results from the sedimentation tank were usually so similar to those from the fish-tank that we have mostly not presented them here.

1. Temperature (Figs. 2, 3)

Temperature varied, as expected, seasonally, diurnally and, slightly, between the different tanks. The small size of the system made it more responsive to air temperature than full size fishponds (Gordin et al., 1990). The diurnal range was about 10° C in winter, and somewhat smaller in summer. The annual range of the daily maxima and minima was from 15° C to 32° C and from 8° C to 27° C in winter and summer, respectively. The temperatures in the polishing tank were more extreme than in the main tanks, due to differences in size and water flow regime. At temperature below 14° C, the fish do not eat (Popper, unpublished). Such temperature, which occurred only during a few weeks in mid-winter, occur less frequently in larger ponds. Yet, raising temperature in mid-winter, by a greenhouse or some other way, should be attempted in commercial systems

fig. 2

fig. 3

2. DO (Figs. 4-6)

Dissolved oxygen was kept at reasonable levels throughout the entire period. In the fish-tank the levels were higher in the morning (due to the lower temperatures and the fish being before feeding) than in the afternoon. Rarely did the DO drop below the safe limit (for *Sparus aurata*) of 4 mg/l or rise to supersaturation (Fig. 6). In the seaweed tanks and the polishing tank, the situation was reversed, as photosynthetic oxygen release elevated DO levels in the afternoon relative to the morning. In this, the seaweed oxygen production compensated for the fish oxygen consumption. Most of the day, DO was supersaturated in the seaweed tanks (Fig. 6).

3. pH (Figs. 7, 8)

The pH levels in the fish-tank were maintained at a comfortable range of 7.3 to 8.6. In the seaweed tanks, the photosynthetic uptake of CO₂ increased the pH by about 0.5-1 unit above that in the fish-tank. In the polishing tank, which received its water mostly from the third seaweed tank (UT3), and which had no inflow through most of the day, the pH level tended to be somewhat higher yet, by another 0.5-1 pH units. However, the fish were never exposed to these damaging levels, as occurs during a summer phytoplankton blooms in intensive fishponds.

4. NH₃

a. Electrode ammonia analyses (Figs. 9, 10)

Most of the daily monitoring, except in the third year, was taken this way. The main feature in the data is the day to day stability, in contrast to the fluctuations observed in phytoplankton based intensive fishponds with comparable feeding rates (e.g., Neori et al., 1989). The quick light-induced uptake of ammonia by the seaweed during the day kept ammonia levels in the whole system at check, usually below 0.1 mM (1.7 mg/l) in the fish-tank. Only when the system did not operate optimally, such as during an intended disconnection of one of the three parallel seaweed tanks (June, 1991), or during other problems (Table 1), did the ammonia level rise above 0.1 mM in the fish-tank, and even then never to the dangerous range above 0.4 mM.

b. Auto Analyzer ammonia analyses (Fig. 11)

Autoanalyzer ammonia analyses were initiated routinely only from the second year. However, some such data exist for May, June, October and November 91 and for January, March, May and July 92. The results can be compared to those in Fig. 10, and show that at least some of the high values generated by the electrode were exaggerated.

fig. 4

fig. 5

fig. 6

fig. 7

fig. 8

fig. 9

fig. 10

fig.11

fig. 12

c. *Electrode vs AA* (Fig. 12)

Simultaneous measurement of ammonia in the same samples by both electrode and AA allowed us to generate a long-term reference curve. The linear regression curve (excluding the three low points below the main body of points) was:

$$Y = 0.92 (+/- 0.012) \times X +/- 14.4 \quad \text{with } R^2 = 0.90 \text{ and } N = 274$$

Clearly, the electrode data can serve as a diagnostic tool. However, their accuracy was far from satisfactory for scientific purposes.

5. *Analyses of NO₂⁻, NO₃⁻, NO₂⁻ + NO₃⁻* (Figs. 13, 14, 15)

Nitrite and nitrate, the products of nitrification, occurred in the system most of the time, at levels of up to 0.23 mM for NO₂ (Fig. 13) and 0.18 mM for NO₃ (Fig. 14). The sum of the two oxidized forms of N (Fig. 15) was around 0.2-0.25 mM most of the time, except following startup of the system in May 92, September 92 and March 93 (Table 1).

6. *TDN* (Fig. 16)

Total dissolved N, which includes the anorganic N compounds above and DON (Dissolved Organic N), showed higher concentrations in FT and SD than in the UT's. In the D tank, TDN levels were by far the lowest in the system. TDN also fluctuated seasonally, for instance in FT, from up to 0.28 mmolar in summer to up to 0.39 mmolar in winter.

7. *DON* (Fig. 17)

The concentration of DON in the system was rather stable, mostly ranging between 0.05 and 0.12 mmolar, or up to 40% of TDN. Its composition has not yet been determined, but according to the literature the DON excreted by marine teleost fish is mostly the amino acid trimethylaminoxide, produced by the fish for osmotic control (Forster and Goldstein, 1969). It is interesting to note that in July 92, with no fish in the system, DON levels did not drop significantly.

8. *PO₄, TDP and DOP* (Fig. 18)

Concentrations of TDP and PO₄, were variable. The low levels, as in Sept. 92, resulted from the system being just stocked with fish, and the levels not yet built up (same as in all other nutrients). Thus, the stable levels were in the range of 20-50 micromolar TDP, and slightly lower for PO₄. DOP levels were rather stable, at about 5 micromolar, or 10% of TDP.

fig. 13

fig. 14

AUTOANALYZER ANALYSES

fig. 15

NO₂+NO₃ CONCENTRATION 91-93

fig. 16

TDN CONCENTRATION 91-93

fig. 17

DON CONCENTRATION 91-93

PO4 CONCENTRATION 91-93

TDP CONCENTRATION 91-93

DOP CONCENTRATION 91-93

B. *THE PILOT SYSTEM*

Figure 19 shows the ammonia levels in the three ponds which participated in this pilot, over the period of September to November. While the ammonia concentration in the conventional ponds, B1, fluctuated widely and reached values above 5 mg l₁, in the fish pond (B2) connected to the biofilter pond (B3) ammonia concentration never reached even 2 mg l₁. Later, with more fish and hence more feed input in the B2-B3 system (Fig. 20), ammonia levels in all ponds increased. Yet in the fishpond connected to the biofilter pond, in spite of much higher feeding rates and the winter season, the ammonia levels were more stable and never reached 4 mg l₁, while in the conventional ponds ammonia concentration went over 5 mg l₁ several times. Fish growth in the two pond system was similar (Popper, unpublished).

C. *HYDROLOGICAL BALANCE*

Tank volumes, evaporation rates and residence times are presented in Table 2. Estimated precision for the volume estimates was 2.2%. The true operating volume was 9200 l less the volume of fish and *Ulva lactuca* plus the undefined volume held in the pipes. Subsequent calculations assume these volumes to be equal. Water residence times were calculated with the above hydrological data and considering the daily water exchange rate of 2000 l and the estimated daily outflow through the polishing tank of 1800 l.

When the system was operating, that is, water WAS flowing in it, the highest salinity (44.50 ppt) was found as expected in the polishing tank. Considering that there was a daily inflow of 2000 liters with salinity 40.80 ppt, and Using the formula:

$$(4) E = F_i * (1 - S_i/S_o)$$

An evaporation rate of approximately 190 liters day⁻¹ was calculated. Loss due to leaks and samples from the system amounts to 41 liters day⁻¹, bringing the total daily water loss to about 231 l.

Figure 19. A time-course of ammonia concentrations in three identically-sized ponds (see Methods section). Pond B1 was a conventional phytoplankton-dependent fishpond with a water exchange rate of half volume d_{-1} , and a feeding rate of 6.5 kg fish pellets d_{-1} . The system of ponds B2 (stocked with fish) and B3 (stocked with the seaweed *Ulva lactuca*), between which water was recirculated at a rate of 400 m³ d_{-1} , also received half of its volume (that is, 100 m³) of clean water exchange, which entered the system via pond B2. Pond B2 received a feeding rate of 10 kg fish pellets d_{-1} .

Figure 20. Same as in Fig. 19, but with higher fish densities and feeding rates in the system of B2-B3 (up to 1800 kg of fish and a feeding rate of 20 kg feed pellets d^{-1}). Polynomial curves were fitted to the data (pond B1, solid line; B2, dashes; B3, dots). The fish biomass in pond B1 was adjusted to receive the same ration of 6.5 kg feed pellets d^{-1} throughout the period).

D. DISCUSSION

The intensive, integrated culture of marine fish with seaweed biofilters took place for two years without the water quality ever deteriorating. Both model and full-size systems maintained reasonable levels of DO, ammonia and pH for the fish. The main principle which enables this system to work so well is that the heterotrophic processes by which the fish and bacteria deteriorate water quality, are counteracted by the photosynthesis of the seaweed. Whereas fish metabolism consumes DO, reduces pH and releases ammonia, the seaweed produce DO and increase pH during the light hours (which is also the time of maximal fish metabolism) and at the same time take up ammonia from the water. The stability and control of the seaweed biofilters, unlike the situation with phytoplankton ponds, allows predictable performance for the integrated system. The main requirement for such performance is that fish metabolism (through feeding rate) is carefully adjusted, with a margin of safety, to the biofilter expected performance. By adjusting the ammonia supply rate to the middle of the seaweed ammonia uptake curve from Cohen and Neori (1991), we have achieved a self-buffered system, where any increase in ammonia supply is restrained by a nearly comparable increase in the seaweed uptake rate.

The seaweed-produced oxygen contributed significantly to the oxygen balance of the fish compartment precisely at the time when it was most needed, several hours following feeding time. Considering a constant difference of 2 mg DO between the seaweed tanks and the fish tank, the water exchange at the hourly residence time of about 2 hrs, would bring into the fish-tank 24 g oxygen/l/d, in addition to the *in situ* aeration. This is significant, considering that 600 g of feed would generate respiration of, at the most, about 600 g of oxygen (Boyd, 1990), or only 14 g /l /d.

The separation of the fish from the algae prevented the fish from experiencing supersaturation of DO, which often leads to the gas bubble disease in the intensive ponds and even in sea cage cultures. It also prevented the fish from experiencing high pH. At the low pH values prevailing in the fish-tank in the afternoon, the time of ammonia maxima (Neori et al., 1989; Krom et al., in prep.), the ammonia was practically all ionized, and hence non-toxic.

That the system is robust and predictable can be seen by comparing any parameter of water quality with the events rundown in Table 1. For instance, the operation of only 2/3 of the seaweed biofilter in June 91 resulted in no effect on the DO, a small decrease in pH, and about a doubling in the morning ammonia concentration. Yet even these higher values were below damaging values (see Wajsbrodt et al., 1991) and were not runaway, that is, they remained stable at the new level and were reduced by the afternoon to the same levels as the month before, when the system had with the full size of the biofilter in operation. Periods of untypically bad weather, with low irradiance and temperatures, resulted in little or no influence on ammonia levels.

The full size integrated system show that the seaweed biofilter allows to intensify fishponds much above the present practice, thanks to the stability of the water quality, without using any more clean water, and with the production of an additional yield of seaweed. In less than a year of operation, the system produced 2.5 tons of fresh seaweed biomass (Neori, 1991; Neori et al., in preparation).

We conclude that the studied concept, the intensive culture of fish in ponds, whose water recirculate through seaweed biofilters, has fulfilled the expectations. It provided stable water quality, allowed high intensification of fish culture, saved use of clean seawater and produced a significant crop of seaweed biomass.

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TABLE 1: Notes on events and occurrences which might have affected the water quality of the system

1991		
1.	May:	regular operation
2.	June:	only 2 UT tanks in use (2/3 of biofilter)
3.	Dec. 31:	installed a heating body in FT to compensate for small size
1992		
4.	Jan. 1-6:	ammonia probe malfunction
5.	Jan. 6:	change ammonia probe
6.	Feb. 7-10:	uncontrolled exchange of clean water (probably too low)
7.	Feb. 10:	pipe cleaning
8.	Feb. 9-12:	DO electrode malfunction
9.	April 5:	removed the heating body from FT
10.	April 6:	fish weighing and readjustment
11.	June 17:	FT vacated and cleaned (put back on line, but without fish because of the outbreak of a disease in the NCM)
12.	June 23-29:	artificial nutrients (NH_4Cl , Na_2PO_4) added daily to support the seaweed growth in the absence of fish
13.	June 30:	added an excessively high quantity of artificial nutrients
14.	July 1-7:	seaweed stock in bad shape, no nutrients added
15.	July 7-10:	system water exchanged with clean seawater
16.	July 19:	excessive exchange of clean seawater
17.	Aug. 3:	FT restocked with fish
18.	Aug. 14:	recirculation pump malfunction
19.	Aug. 16:	fish disease discovered
20.	Aug. 17:	FT put off line for chemical treatment of the disease
21.	Aug. 21:	fish have no appetite, fed 2/3 of usual ration
22.	Aug. 23:	fish have no appetite, fed 2/3 of usual ration
23.	Aug. 24-31:	FT off line for disease treatment
24.	Aug. 27:	UT receive artificial nutrients
25.	Oct. 7:	Following thorough cleaning, a new stock of <i>Ulva</i> has been brought from the sea
26.	Nov. 2:	FT put off line for chemical treatment of fish disease
27.	Nov. 18:	DO electrode malfunction
28.	Nov. 19:	recirculation pump malfunction due to clogging
29.	Nov. 30:	pipe cleaning
30.	Dec. 2:	a cloudy day
31.	Dec. 10:	a heating body placed in FT
32.	Dec. 12:	pipe cleaning
33.	Dec. 16:	a very cloudy day

34. Dec. 21: a pump malfunction caused low water level in FT
 35. Dec. 21-24: rapid exchange with clean seawater until pump is repaired
 36. Dec. 24: samples were taken at 10:15, instead of 8:30
 37. Dec. 26: malfunction of clean water meter, resulting in rapid exchange with clean seawater
 38. Dec. 29: a very cloudy day
 39. Dec. 30: the 2000 l of clean seawater was given only at 20:00 due to an experiment

1993

40. Jan. 4: a very cloudy day
 41. Jan. 5: rain and clouds; feed given only at noon
 42. Jan. 7: clouds and rain; pipe cleaning
 43. Jan. 7-10: feeding at noon
 44. Jan. 13: pipe cleaning
 45. Jan. 22: air system breakdown; no feeding; rapid exchange with clean seawater
 46. Jan. 27: the 2000 l of clean seawater was given only at 20:00 due to an experiment
 47. Feb. 3: a new DO electrode put in use
 48. Feb. 3: a cloudy day; pipe cleaning
 49. Feb. 4: clouds and rain
 50. Feb. 21: the 2000 l of clean seawater was given only at 20:00 due to an experiment
 51. Mar. 11: fish harvest
 52. Mar. 30: pipe cleaning
 53. Apr. 13: removed the heating body from FT
 54. Apr. 13: pipe cleaning
 55. May 3: water recirculation stopped (14:00-19:15) for an experiment
 56. May 18: Ulva sporulation and partial fragmentation
 57. Jun. 3: 2500 l of clean water instead of the usual 2000 l
 58. Jun. 8: Ulva sporulation and partial fragmentation
 59. Jun. 9: fish transfer, excess input of clean SW to UT's
 60. Jun. 10: FT restocked with fish, system back to normal operation

TABLE 2: The hydrology of the experimental system.

Component	Volume (l)	Evaporation Rate (l d ⁻¹)	Residence Time (h)
Seaweed Tank (each)	2130 (x3)	56.4 (x3)	6.5-7.9
Fish Tank	1790	48.0	1.8-2.2
Sedimentation Tank	400	11.0	0.4-0.5
Polishing Tank	600	24.0	7.9
Whole System	9200	190-253	4.6 days

IV. FIGURE LEGENDS

Figure 1. A schematic configuration of the integrated system for the culture of fish and seaweed. Tanks: A, fish-tank (FT); B-D, seaweed tanks (UT1, UT2, UT3, respectively); E, polishing tank (D); F, sedimentation tank (SD); G, recirculation pump. Major water flows: 1, inflow of clean seawater; 2, sludge drainage of SD tank; 3, main outflow.

Figures 2-18. See figure titles

Figure 19. A time-course of ammonia concentrations in three identically-sized ponds (see Methods section). Pond B1 was a conventional phytoplankton-dependant fishpond with a water exchange rate of half volume d^{-1} , and a feeding rate of 6.5 kg fish pellets d^{-1} . The system of ponds B2 (stocked with fish) and B3 (stocked with the seaweed *Ulva lactuca*), between which water was recirculated at a rate of $400\text{ m}^3\text{ d}^{-1}$, also received half of its volume (that is, 100 m^3) of clean water exchange, which entered the system via pond B2. Pond B2 received a feeding rate of 10 kg fish pellets d^{-1} .

Figure 20. Same as in Fig. 19, but with higher fish densities and feeding rates in the system of B2-B3 (up to 1800 kg of fish and a feeding rate of 20 kg feed pellets d^{-1} . Polynomial curves were fitted to the data (pond B1, solid line; B2, dashes; B3, dots). The fish biomass in pond B1 was adjusted to receive the same ration of 6.5 kg feed pellets d^{-1} throughout the period).

FINAL REPORT

Chapter II: FISH AND SEAWEED YIELDS DURING TWO YEARS OF NEARLY CONTINUOUS OPERATION

During the operation of the model system, the seaweed was harvested from the three *Ulva* tanks and the polishing tank weekly or biweekly (see Chapter I for technical details). The fish were weighed and their biomass adjusted less frequently, to avoid excess stress. The result was that often the fish were under-nourished, as the feed daily input remained 0.6 kg throughout the period to allow the analysis of the water quality data. Hence, fish yield can be expected to be below the standard in a normally operated system, in which feed ration is adjusted frequently to fish biomass. In this short chapter we report on the actual outputs (harvests) of fish and seaweed from the model integrated system described in Chapter I.

I. Fish Yield

The fish output data are presented in Fig. 1. Negative numbers indicate fish-stocking. The slope of the overall fish-output curve is 0.14 kg d^{-1} , which is comparable to a food conversion ratio (FCR) of 4.3 kg feed per kg of harvested live fish. This value is at the high end of FCR values, scattered near the average of 3 achieved in conventional intensive ponds (Shpigel et al., 1993). Underfed fish can be expected to have high FCR values, as they use a large fraction of the feed intake for body maintenance instead of growth. In units of pond volume, considering the whole 9000 l in the recirculating system (including the FT, SD and UT's but not the polishing (D) tank, the yield is about $15 \text{ g m}^{-3} \text{ d}^{-1}$, or comparable to an annual harvest of 55 t ha^{-1} .

II. Seaweed Yield

The seaweed fresh yield data are presented in Fig. 2. The overall yield, spanning nearly two years and 87 harvests, averaged at 1.15 kg d^{-1} , or about $115 \text{ g m}^{-2} \text{ d}^{-1}$. On an annual basis, this yield equals $1150 \text{ kg ha}^{-1} \text{ d}^{-1}$, or $420 \text{ t ha}^{-1} \text{ y}^{-1}$. During spring and summer, the yield was much higher, at $1.9\text{-}2.4 \text{ kg d}^{-1}$, while in mid-winter it dropped to only $0.6\text{-}0.9 \text{ kg d}^{-1}$.

III. N- yield in Seaweed

The food input to the system was 214 kg y^{-1} , containing 13.36 kg of nitrogen (at 6.1% N-content). The seaweed N-analyses ranged from 2.5-6% of DW, and DW averaged 17% of FW. These numbers and the total harvest from above put the range for the fraction of N-input harvested as seaweed biomass at between 13.4% to 32.0% of the total annual N-budget, a range which covers the ranges for the individual daily N-budgets calculated in Chapter III below.

IV. In summary, the system achieved respectable yields for both fish and seaweed although its mode of operation was intended not for maximizing these yields, but rather to allow accurate measurement and interpretation of water quality parameters. Per kg of feed input, nearly 2 kg of seaweed were harvested, as compared to 0.23 kg of fish.

Figure 1

Figure 1

SEAWEED YIELD Integrated fish—Ulva system

Figure 2

DAYS OF OPERATION

+ Yield ◇ Cumulative yield — Regressions

Nov. 93

Aug. 93

Apr. 93

Jan. 93

Aug. 92

Apr. 92

Jan. 92

Oct. 91

Figure 2

FINAL REPORT

Chapter III: NITROGEN AND PHOSPHORUS CYCLING AND TRANSFORMATIONS IN A PROTOTYPE "NON-POLLUTING" INTEGRATED MARICULTURE SYSTEM, EILAT, ISRAEL.

I. ABSTRACT:

An intensive mariculture system for the growth of seabream has been developed, which uses seaweed (*Ulva lactuca*) to remove dissolved nutrients (N and P) from the circulating water. This allows water to be safely recycled to the fish and results in a comparatively "non-polluting" final effluent. As with other mariculture systems, only ~25% of the food-N was used for fish growth. The dominant processes affecting N within the system were excretion by fish of ammonia-N (30%), DON (30%) and faeces-N (10%), rapid transformation of DON to ammonia-N (16%) in the fish tank and uptake of ammonia-N (17-39%) by the seaweed (all percentages expressed relative to food input). Nitrification (10%) competed with the seaweed for the available ammonia-N. There was only minimal uptake of nitrate by the seaweed. Anoxic conditions developed in the sedimentation tank. Denitrification (5%) and bacterial ammonification, including sulphate reduction, were observed. The system showed only minor seasonality, probably because the major processes (food input and *Ulva* uptake) have only a slight dependence on temperature. Our quantitative understanding of the processes affecting N within the system was confirmed by a parallel study in which a computer simulation model was developed which was able to predict well the observed changes in ammonia-N, TOxN and *Ulva* growth. There was an excess of phosphorus in the food supplied. It was removed by the seaweed (9-21%) but to a lesser degree than ammonia-N. The residual OM in the sedimentation tank was enriched in P, probably because of the presence in the food of bone meal which is resistant to bacterial decay. The final effluent discharged contained 20-27% of the N supplied (and 39-47% of the P) which is less than half the N discharged from conventional mariculture technology.

II. INTRODUCTION:

The development of sustainable integrated agricultural technologies is important if we are to feed an ever growing population with minimum damage to the environment. Mariculture is one of the last major untapped food resources. Recently there has been a considerable growth in this agricultural sector in western Europe, North and South America, mainly the culture of salmon in sea cages.

Fish grown in mariculture systems retain only 20-30% of the food supplied as body flesh (Porter et al., 1987, Hall et al., 1992). The remaining 70-80% is excreted as either dissolved or particulate nutrients. These waste products have the potential to cause a variety of pollution problems, including reduction in oxygen content and ultimately anoxia, changes in ecosystem structure, damage to fragile ecosystems such as coral reefs (Banner, 1974; Tomascik and Sander, 1985; Bell et al., 1985) and increased frequency of toxic dinoflagellate blooms (Watanabe, 1991). Mariculture systems operating in this way are also wasteful of energy, since the excreted nutrients are high-quality and high-energy food (such as reduced N) usable for other commercially valuable marine organisms. These problems have long been recognized by freshwater aquaculturists and a variety of integrated systems have been developed such as carp-mulberry bush (silkworm) culture

in China, carp-rice-pig culture in the Far East (Pillay and Dill, 1979; Woynarovitch, 1979; de la Cruz et al., 1992) and integrated fish culture and vegetable growth (Rakocy and Hargreaves, 1993).

Over the past decade, scientists at the National Center for Mariculture in Eilat, Israel have set out to develop integrated mariculture systems which exploit the natural advantages of the local climate (sunshine and heat; Gordin et al., 1981). Previous attempts have used phytoplankton to remove excess nutrients from the fishpond water and oysters as biofilters to provide an extra commercial crop (Gordin et al., 1981, Krom and Neori, 1989, Gordin et al., 1990). However, in such systems the phytoplankton are subject to major and uncontrollable blooms and crashes caused by microflagellate grazing (Krom et al., 1989). These fluctuations cause large changes in water quality conditions, particularly in ammonia and dissolved oxygen, which can result in fish mortalities (Krom et al., 1985). Such systems also export most of the nutrients from the fishpond as a mixture of dissolved and particulate nutrients, the proportions depending on the phytoplankton bloom and crash status at the time (Krom and Neori, 1989; Neori and Krom, 1991). Under such conditions, the effluent waters from the ponds are unreliable as a food source for the second crop, bivalves, and in turn bivalves are not dependable as biofilters to clean the pollution from the system (Shpigiel et al., 1993).

The use of seaweed as a biofilter was first suggested by Ryther et al., (1975). According to them, a biofilter system based on seaweed had the potential of producing stable water quality conditions for the fish and a final effluent low in nutrient waste products.

The system reported here represents a pilot study of such a sustainable mariculture system which cultures gilthead seabream (*Sparus aurata*) in a pond coupled with tanks of the seaweed *Ulva lactuca*. Growing fish in intensive culture loads the pond water with high fluxes of nitrogen and phosphorus. These sustain a variety of microbial and biological processes, characteristic of hypertrophic systems, which interact with one another and compete for the available nutrients (Barica and Mur, 1979). In this study we set out to determine which processes were occurring within this pilot scale system, what was their relative magnitude throughout an annual cycle and how the different processes compete for available nutrients. These questions were addressed by means of a series of total nutrient budgets designed to identify and quantify the significant processes within the system and how they change with time. Specific experiments were carried out to locate these processes within the system and to estimate their rates.

In a parallel study, a computer simulation model of the system was developed. It represented a quantitative hypothesis about the processes controlling N in the system based on this study and how they interact (Ellner et al., submitted.). The model was tested using two sets of independent data. The quality of the fit of the measured data to the model's predictions provided an estimate of how well we understand the principle processes within the system.

III. METHODS:

A. *Description of system:*

The fish-seaweed biofilter system consisted of four fiberglass tanks (2.5 m³; 3.3 m² each), one for fish containing 1790 l of seawater and the other three for seaweed containing 2130 l each (Figure 1). Water was circulated by an electric pump from the fishtank via a small (400l) sedimentation tank, to the three seaweed (*Ulva*) tanks arranged in parallel. Water then returned to the fish tank by gravity. Screens at the end of each *Ulva* tank kept the seaweed from washing out. The whole system contained approximately 8600l of seawater which was circulated internally at a rate of 800-1000 l/hr. Each day, 2000l of fresh seawater was pumped into the system in two

pulses each of 1000l at 08:30 and at 16:30. There was a simultaneous outflow of 1800l (input less evaporation and sampling; Neori et al., in prep., see earlier in this report) which flowed out via a 600l polishing tank to the sea. A small volume (up to 10l) of water containing sludge was drained daily from the sedimentation tank.

In May of 1991, the fish tank was stocked with approximately 23 kg of young *Sparus aurata*. The fish were initially fed daily (08:30) 600 g of the pelleted diet described in Krom and Neori, (1989). Fish numbers (and biomass), and feeding rate were adjusted periodically so that a food ration of 600 g could be maintained throughout the study period. The fish had to be replaced several times during the running period, either because of excessive size or because of fish disease (Neori et al., in prep., see earlier in this report). The fish tank was shaded to limit photosynthetic activity and was aerated and agitated via holes in the tank base. The Ulva tanks were stocked with *Ulva lactuca* at an initial stocking density of 3 kg per tank, (the optimum, based on Neori et al., 1991). The seaweed was harvested weekly and immediately restocked at the original density. The experimental system was run in this mode from April, 1991 until October, 1993.

B. *Sampling and analysis:*

A series of six intensive 24 hour sampling periods for total nutrient budgets were carried out between October 1991 and May 1992. Each sampling began at 08:30 and was repeated every 3 or 4 hours until the following morning. Water samples were taken from the fish tank (FT), the sedimentation tank (SD), each of the three seaweed tanks (UT1, UT2 and UT3) and the polishing tank (D) (see Figure 1 for sampling locations). The fresh seawater inflow (IF) was sampled once per sampling period in the morning. Each water sample consisted of one liter for dissolved and particulate matter measurement. In addition the sump of the sedimentation tank was drained for several seconds and the entire water sample (with its sediment) collected.

The samples for dissolved nutrient determination were filtered through acid-washed GF/F filters within 1 h of sampling. The filtrate was divided into several subsamples. One was immediately analyzed or stored in a dark refrigerator at 4°C, typically for a day (after a maximum of 3 days all these analyses were completed). Analyses were made by the automated methods summarized in Table 1. The second subsample was immediately placed in a freezer (-15 to -20°C). Although preservation tests showed that frozen samples gave similar results to the unfrozen samples, all the data used in this study were from unfrozen samples except for the very few occasions when data were obviously inconsistent with adjacent data (in time and/or space), in which case the results from the frozen samples were used. Subsamples for total dissolved nitrogen and phosphorus were also refrigerated in the dark at 4°C. The method used combined basic oxidation for N and acidic oxidation for P, in one sample bottle as described in detail in Neori et al., (in prep., see earlier in this report). All dissolved samples were analyzed in duplicate. Samples for particulate C, N and P were filtered on pre-ashed GF/F filters. The samples were then placed in aluminum foil and frozen. Before analysis the samples were vacuum dried in a Virtis Model 25 SRC vacuum dryer. These samples were also analyzed in duplicate. The analytical methods used together with the relevant performance characteristics are given in Table 1.

In addition, during several of the sampling periods, other aliquots were filtered for chlorophyll determination, preserved for bacterial cell-counts (using DAPI fluorescent staining) and also used for immediate live microscopic hemacytometer counts of phytoplankton and protozoa. Chlorophyll was extracted from the glass-fiber filters in 90% acetone and analyzed by the spectrofluorometric method described in Neveux et al., (1990), using a Perkin Elmer LS 5b Spectrofluorometer connected to an IBM-compatible computer.

The seaweed tanks including the polishing tank were harvested weekly, as described in Neori et al., (in prep., see earlier in this report), the harvest was weighed and the tanks were restocked with 3 kg of seaweed each. The wet yield was gained amount. In order to determine the dry yield of seaweed in the system, a subsample of the harvested seaweed was weighed, rinsed, dried in an oven at 70°C, and reweighed. An aliquot of this dried subsample was subsequently analyzed for C, N and P as described in Table 1. The final polishing tank, was also harvested and sampled weekly, in a similar manner. The slurry which was drained from the sedimentation tank was oven-dried (70°C), and the quantity of the collected particulate matter measured. A subsample of the dry material was analyzed for C, N and P content.

To determine the total nutrient budget (Tables 2 and 3), the total input was calculated from 600g of food x measured N and P content of the food. The amount of N and P taken up as fish growth was calculated from N retention tables measured for *S. aurata* in Eilat using the same food as that used in this study (Lupatch, unpublished). The daily *Ulva* yield was calculated from the measured weekly yield assuming a constant daily growth rate. Sludge, removed from the system, was measured directly during each of the intensive sampling periods. The nutrient outflow was calculated from the average concentration of dissolved and particulate nutrients measured in the *Ulva* tanks at the times of water outflow x measured flow rate. The denitrification rate was estimated from the values determined during the no-flow experiment (see below). The deficit was the input minus the sum of the outflows from the system.

In May 1993 an experiment was run in which the flow of water in the system was entirely stopped for 5.25 hours (14:00 -> 19:15). Water was sampled in the fish tank, sedimentation tank and *Ulva* tanks initially and then every hour from 15:15 until 19:15. The samples were processed in the same way as described above for the 24 hour sampling periods.

C. *Operating conditions of the system:*

Water quality was monitored throughout the sampling period on a regular basis (Neori et al., in prep., see earlier in this report). In brief, the weekly average temperature in the system ranged from 23°C in summer to 14°C during winter, with diurnal variation of up to 10°C. Light levels over the year, ranged from 120 to 190 Einsteins m⁻² d⁻¹. The salinity in the system increased from 40.3 ppt (the inlet seawater) to 44.6 ppt in the final polishing tank. Dissolved oxygen levels in the fishtank ranged from 4 to 9 mg/l, with diurnal fluctuations of 1-2 mg/l. In the *Ulva* tanks the dissolved oxygen levels ranged from 7 to 12 mg/l, with diurnal ranges of up to 4 mg/l. Particulate chlorophyll *a* levels (excluding that in seaweed fronds) ranged from 1 to 4 fg l⁻¹.

IV. RESULTS:

The input of nutrients in food was constant throughout the sampling period at 2.53 moles N/d and 0.290 moles P/d (Table 2 and 3). The N uptake by fish ranged from 19-26% of the input with a slightly higher uptake in the warmer months (May-Nov.) than in winter (Jan.-March). Specific growth measurements were made only occasionally during this sampling period so as not to disturb the fish unduly (see an earlier chapter in this report). Using the measured elemental composition of the fish (Lupatch, unpublished), the two data sets were consistent with one another. *Ulva* yield was 0.44-0.99 moles N/d which represented 17- 39% of the N input. The amount of P retained by the *Ulva* (0.27-0.61 moles P/d) was 9-21% of the total input, less as a percentage of total input than N. The removal of N to waste in the sedimentation tank was 1.4-3.5% of the total input. The relative fraction of P sedimented out in this tank was greater, at 4.5-13.8%.

The main water quality parameter that this system was designed to control was ammonia-N in the fishpond. At least during the days covered by the present data set, the levels of ammonia-N within the system was maintained below 120 micromolar in the fish tank and below 30 micromolar in the Ulva tank (Fig. 2). The peak of ammonia-N in the Ulva tank was at the end of the night while the peak of ammonia-N in the fish tank was at 15:00, 8 hours after feeding. Neori et al., (in prep., see earlier in this report) showed that this pattern of tight control of ammonia-N was typical of values obtained throughout the year. The amount of dissolved ammonia-N exported from the Ulva tank was low, <0.04 moles N/d or $<1.4\%$ of the input N flux to the fish tank. The export of DON was 0.05-0.15 moles/d (1.9-5.9%). There was a steady flux of the system (Table 2). However the relative proportion of nitrate to nitrite increased systematically throughout the sampling period (Fig. 3). The export of particulate organic N (PON) was steady all year at $0.2 (+/- 0.04)$ moles N/d. Neori et al., (in prep., see earlier in this report) measured the chlorophyll-a content for this particulate matter as 1-4 fg/l. If one assumes a similar mass ratio for N/Chl-a of 4.4 to that determined previously for phytoplankton in fish ponds in Eilat (Krom and Neori, 1989) then it is estimated that only 0.57-2.2 mmoles N/d was exported as phytoplankton and microscopic seaweed fragments. A further quantity of <0.1 mole N/d of the particulate matter was identified as bacterial cells. The microscopically- determined biomasses of heterotrophic eucaryotic organisms, such as protozoa, never exceeded 0.1 mole N /d. This export of identifiable microbes was small, compared to the seaweed yield of about 0.6 moles N/d exported from the system.

While it was suspected that denitrification might be a significant process within the system it was not possible to quantify it using the data from the total N budget. It was however found that during the no-flow experiment, denitrification in the sedimentation tank was a significant process in the system. We used the rate determined from the no flow experiment (0.15 moles N/d; see below) for all the sampling periods.

The principal export of P from the system was as phosphate (22-31%) with smaller amounts of DOP (1-13.7%) and particulate organic P (4.3-6.8%). The amount exported, expressed as a percentage of input, was 39-47% which was greater for phosphate than for ammonia-N.

Having completed our best estimate of the total N and P budgets, we found a consistent deficit of approximately 20% (Table 2 and 3). Given the large number of data, each with their own sampling and analytical errors, we consider this to be an acceptable deficit. The only process which might contribute substantially to the N-deficit and which we did not include in the budget was coupled nitrification-denitrification.

Nutrient budgets for individual chemical species were calculated for each tank every 3 hours during the 24 hour sampling period. The change in flux represented the net biogeochemical process occurring in that tank. We found that the rate of water recirculation was so rapid that the precisions of the different analyses were not sufficient to allow quantitative estimates of processes in each tank to be made, except for the production of ammonia-N by fish excretion in the fish tank and uptake of ammonia-N by Ulva. To solve this problem an experiment was conducted in May 1993 in which the flow of water in the system was entirely stopped for a few hours. There was a linear change in nutrient content over the first 3.25 hours of the experiment in the fish tank and over the first 4-5 hours in the sedimentation and Ulva tanks (Fig 4-6). The net rate of change of dissolved nutrient was obtained from a regression calculated for the linear portion of the experiment. The ammonia-N change in the fish tank (14:00 -> 17:15) was 45 mmoles/hr (Fig 4). Porter et al., (1987) showed that the excretion rate of ammonia-N by *S. aurata* depends on the time since the fish were fed. The fish in this experiment were fed at 08:30 and after we applied a correction based on Porter et al.'s data the calculated ammonia-N accumulation was 1.87 moles N/d, or 74% of the N-input. During the same time period only 15 mmoles N/hr were accumulated as DON in the fish tank. Porter et al., (1987) did not determine time dependant relationship of DON excretion from

the fish and so a correction of the type made for ammonia-N was not possible. The accumulation rate of DON in the fish tank was thus estimated to be 0.36 mmoles N/d. This would become 0.56 mmoles N/d if the same correction was applied as was done for ammonia-N excretion. There was an increase in nitrate and nitrite (TOxN) in the fish tank of 6.4 mmoles N/h. No evidence existed for any anaerobic pockets within this tank even under no-flow conditions, and thus this change corresponds to a nitrification rate of 0.17 moles N/d.

Ammonia-N accumulated in the sedimentation tank at a rate of 8.6 mmoles N/h during the first 4 hours of the no-flow experiment, equivalent to 0.21 moles N/d (Fig 5). When the sedimentation tank was drained, a strong smell of hydrogen sulphide arose from the "drainage". The accumulation of phosphate within this tank was closely correlated with ammonia-N (Fig 5). The observed rate of 0.92 mmoles P/h was equivalent to a daily rate of 0.022 moles P/d. TOxN in the sedimentation tank decreased linearly over the first 4.25 hours of this experiment at a rate of -4.8 mmoles N/h which is equivalent to a rate of -0.12 moles N/d. The only process which could be responsible for such a decrease was denitrification. This is likely to be an important process in this tank since there is a high concentration of nitrate within the tank (~90 micromolar initially, decreasing to <30 micromolar after 4.25 hours) and there were anaerobic pockets containing labile organic matter in the tank. The observed denitrification rate in the sedimentation tank is probably a net rate for nitrification + denitrification. We expect that nitrification was also occurring in the sediment tank, whose upper portion was kept constantly aerobic by the inflow of aerated water from the fish tank, and we know that nitrification was occurring in all other aerobic parts of the system where ammonia-N was present. If we assume that the nitrification rate is proportional to the surface area of the tank wall in contact with water, then based on the rates in the fish and seaweed tanks we estimate a nitrification rate of 0.03 moles N/d. Thus we estimated that the denitrification rate in the sedimentation tank was 0.15 moles N/d. This value was used in the budget calculations and in the computer simulation model (Ellner et al., submitted., see later in thi).

The accumulation of TOxN in the Ulva tanks for each of the three tanks (Fig 6) was 2.8 mmoles N/tank/h (0.2 moles N/d for the three tanks together). The ammonia-N concentration within the tanks ranged from 1-73 micromolar. At these ammonia-N levels and for the time period considered, the uptake of TOxN by Ulva was insignificant (Neori et al., in prep.). We can thus consider this rate of TOxN accumulation to be the nitrification rate in those tanks.

V. FIGURE LEGENDS:

- Figure 1: A schematic diagram of the fishpond-seaweed biofilter system with arrows showing the direction of water flow. The sampling locations are shown.
- Figure 2: Ammonia-N concentration in a) fish tank and b) Ulva tank during 24h samplings. Each sampling was started at 8:30h.
- Figure 3: Net nitrification in the system as measured by the net daily export of oxidised N from the system:
- Figure 4: Accumulation of ammonia-N, DON and Total oxidised-N in the fish tank during the no-flow experiment. Sampling was started at 08:30h.
- Figure 5: Accumulation of ammonia-N, phosphate and Total oxidised-N in the sedimentation tank during the no-flow experiment. Sampling was started at 08:30h.
- Figure 6: Accumulation of Total oxidised-N in each of the three Ulva tanks during the no-flow experiment. Sampling was started at 08:30h.
- Figure 7: Pie diagrams showing the proportion of N retained and exported as pollution from a seacage system, a fishpond- phytoplankton system (bloom and crash conditions), and the fishpond-seaweed system with and without the polishing step.

Table 1: Principal analytical methods used in this study together with their performance characteristics.

Determinand	Coefficient of variation	Method used
Total ammonia-N	2.0%	Krom et al (1989)
Total oxidised nitrogen	0.3%	Krom et al (1989)
Nitrite		Krom et al (1989)
Phosphate	0.9%	Krom et al (1989)
Total dissolved nitrogen	8% at 60 micromolar	Persulphate oxidation followed by autoanalyser method for T.Ox.N. (Neori et al. in prep.)
Total dissolved phosphorus	13% at 30 micromolar	Persulphate oxidation followed by autoanalyser method for phosphate (Neori et al. in prep.)
Particulate and solid N	7.6%	Carlo-Erba CHN analyzer
Particulate and solid P	3.8%	Roasting at 450C followed by extraction into 1M HCl (Aspila et al., 1976)

Table 2:
Table of Nitrogen outputs from the fishpond – seaweed system
(Data from 24 hour samplings)
Input flux for all samplings was 2.53 molesN/d
a) Data given MolesN/d

DATE	FISH GROWTH	ULVA YIELD	SEDIMENT TO WASTE	NUTRIENTS OUTFLOW FROM ULVA TANKS						
				AMMONIA	NITRATE	NITRITE	D.O.N.	PART. NIT.	DENITRIF. RATE	DEFICIT
OCTOBER A	0.63	0.68	0.04	0.02	0.03	0.29	0.08	0.19	0.15	0.43
OCTOBER B	0.63	0.58	0.04	0.02	0.02	0.24	0.07	0.19	0.15	0.59
NOVEMBER	0.66	0.58	0.04	0.02	0.02	0.25	0.15	0.19	0.15	0.47
JANUARY	0.53	0.60	0.04	0.04	0.09	0.16	0.08	0.29	0.15	0.56
MARCH	0.48	0.44	0.09	0.01	0.14	0.10	0.10	0.19	0.15	0.83
MAY	0.61	0.99	0.04	0.02	0.20	0.05	0.05	0.16	0.14	0.29

b) Data given as percentage of input flux

DATE	FISH GROWTH	ULVA YIELD	SEDIMENT TO WASTE	NUTRIENTS OUTFLOW FROM ULVA TANKS						
				AMMONIA	NITRATE	NITRITE	D.O.N.	PART. NIT.	DENITRIF. RATE	DEFICIT
OCTOBER A	25.0	26.8	1.4	0.6	1.2	11.6	3.3	7.4	5.9	16.8
OCTOBER B	25.0	23.1	1.4	0.8	0.9	9.5	2.6	7.4	5.9	23.4
NOVEMBER	26.0	22.9	1.4	0.9	0.9	10.0	5.9	7.4	5.9	18.8
JANUARY	21.0	23.8	1.5	1.4	3.3	6.4	3.2	11.5	5.9	22.0
MARCH	19.0	17.2	3.5	0.4	5.7	3.8	4.1	7.4	5.9	32.9
MAY	24.0	38.9	1.4	0.6	8.1	1.9	1.9	6.4	5.4	11.3

Table 3:
Table of Phosphorus outputs from the fishpond—seaweed system
(Data from 24 hour samplings)
Input flux for all samplings was 0.290 molesP/d
a) Data given in MolesP/d

DATE	FISH GROWTH	ULVA YIELD	NUTRIENTS OUTFLOW FROM ULVA TANKS		
			SEDIMENT TO WASTE	PHOSPHATE	DEFICIT
OCTOBER A	0.075	0.033	0.015	0.091	0.018
OCTOBER B	0.075	0.033	0.020	0.068	0.011
NOVEMBER	0.078	0.030	0.020	0.070	0.018
JANUARY	0.064	0.027	0.029	0.064	0.003
MARCH	0.058	0.032	0.040	0.065	0.009
MAY	0.073	0.061	0.013	0.066	0.040
					0.013
					0.025

b) Data given as percentage of input flux

DATE	FISH GROWTH	ULVA YIELD	NUTRIENTS OUTFLOW FROM ULVA TANKS		
			SEDIMENT TO WASTE	PHOSPHATE	DEFICIT
OCTOBER A	26.0	11.2	5.2	31.3	4.7
OCTOBER B	26.0	11.2	6.9	23.4	3.7
NOVEMBER	27.0	10.5	6.9	24.1	4.8
JANUARY	22.0	9.4	10.0	21.9	1.1
MARCH	20.0	10.9	13.8	22.5	3.0
MAY	25.0	21.1	4.5	22.7	13.7
					6.1
					6.1
					6.1
					6.8
					6.1
					4.3
					15.5
					22.8
					20.7
					28.8
					23.8
					8.7

Table 4: Measured and calculated N-uptake and seaweed yield
Units: Moles N/ system/d

Experiment:	Oct a 91	Oct b 91	Nov 91	Jan 92	Mar 92	May 92
gross measured ammonia removal in UT's	0.73	0.80	0.74	0.83	0.82	1.32
Ulva harvest	0.68	0.58	0.58	0.60	0.44	0.99
Expected NH ₃ uptake in UT's from Cohen & Neori, 1991	0.81	0.96	0.98	0.82	0.71	0.87
System (UT's +FT +SD) nitrification(from Table 2)	0.32	0.26	0.27	0.24	0.24	0.25
Total measured NH ₃ uptake by system (Ulva harvest +UT+FT+SD nitrification)	1.0	0.84	0.85	0.84	0.68	1.24

Figure 1: A schematic diagram of the fishpond-seaweed biofilter system with arrows showing the direction of water flow. The sampling locations are shown.

-Figure 2: Ammonia-N concentration in a) fish tank and b) Ulva tank during 24h samplings. Each sampling was started at 8:30h.

Figure 3: Net nitrification in the system as measured by the net daily export of oxidised N from the system:

Figure 4: Accumulation of ammonia-N, DON and Total oxidised-N in the fish tank during the no-flow experiment. Sampling was started at 08:30h.

Figure 5: Accumulation of ammonia-N, phosphate and Total oxidised-N in the sedimentation tank during the no-flow experiment. Sampling was started at 08:30h.

Figure 6: Accumulation of Total oxidised-N in each of the three Ulva tanks during the no-flow experiment. Sampling was started at 08:30h.

Figure 7: Pie diagrams showing the proportion of N retained and exported as pollution from seacage system, a fishpond- phytoplankton system (bloom and crash conditions), and fishpond-seaweed system with and without the polishing step.

VI. DISCUSSION:

A. Fish tank:

As in most intensive aquaculture systems, the major source of nutrients was from the fish food after it was excreted by the fish. The input of nutrients with the water inflow was negligible. 74-81% of the food-N was excreted as ammonia-N, dissolved organic nitrogen or as faeces with the remainder retained by the fish for growth. Similar values for the percentage of fish food retained for growth by *S. aurata* were obtained in laboratory experiments (Porter et al., 1987) and in measurements on fish held in ponds and sea cages (Porter et al., 1986; Krom and Neori, 1989). These values are similar for the retention of N by other species of fish used in mariculture such as salmon, trout and seabass (Steffens, 1981, Alexis et al., 1986, Hidalgo and Alliot, 1988). Thus it is an intrinsic characteristic of mariculture, that even when operated at maximum efficiency for growth of fish, large quantities of nutrients are exported from the fish holding tank or cage.

Porter et al., (1987) estimated that ~10% of the N supplied to *S. aurata* in the food was excreted as faeces with approximately equal amounts of the remaining 60% being excreted as ammonia-N (30%) and as DON (30%). In the no-flow experiment, most of the dissolved N flowing out of the fish tank was as ammonia-N (74% of the N supplied). This included the fraction accumulated as ammonia-N (67%) and the increase in TOxN due to nitrification (7%). By contrast only 14% of the nitrogen was exported as DON. Similarly during all the 24 hour experiments the flux of DON from the fish tank was ~26% of the total dissolved N flux compared to the 50% predicted by Porter et al., (1987). It was concluded that bacterial ammonification was occurring in the fish tank which rapidly converted DON to ammonia-N. A similar rapid conversion of DON to ammonia-N was observed by Krom and Neori (1989) in experiments on full scale (100 m³) intensive fishponds. This can be of considerable practical importance since ammonification can convert DON, which is often unutilizable by seaweeds (Lobban et al., 1985), to ammonia-N, the favored N-source of many seaweeds. Details of these rapid transformations involving DON are not well studied in hypertrophic systems but as these data show they may be very important.

B. Sediment tank:

The sedimentation tank was included in the system to trap particulate matter, particularly fish faeces, while maintaining a flow of oxygenated and nutrient-rich water between the fish tank and Ulva tank. It was predicted from the results of Porter et al., (1987) and Krom and Neori (1989) that 10% of the N budget should be found as faeces. In fact only about 3% was found as sedimented material in this tank. The sediment was drained from the tank once per day which meant that particulate organic matter (POM) accumulated for an average of 12 hours and a maximum of 24 hours.

In the no-flow experiment, 0.2 Moles ammonia-N/d were produced within the sedimentation tank (Fig. 5). If it is assumed that all the ammonia-N produced in the sedimentation tank is the result of bacterial ammonification of POM (there was no significant net change in DON), then the total amount of POM in the tank (measured as drained POM plus respired) was 0.24 Moles N/d which is close to the amount of faeces predicted by Porter et al., (1987) as being produced by the fish.

Although the input and outflow of the sedimentation tank remained permanently oxic (Neori

et al., in prep., see earlier in this report, and data not shown), parts of the sedimentation tank became anoxic, as evidenced by a strong smell of hydrogen sulphide in the drained material. During the no-flow experiment (May 1993), there was a decrease in TOxN equivalent to 0.12 Moles N/day, which (when corrected for our best estimate for nitrification in the upper layers of this tank) corresponded to a denitrification rate of 0.15 Moles N/d. This rate was equivalent to a loss of nitrogen (N_2) from the system equivalent to 5% of the total N supplied (Table 2, Fig 5). This is probably a minimum value for the entire system. If there are any other pockets of anoxia, such as in the connecting pipes or in the bottom of the Ulva tanks, we would expect denitrification to occur there as well. Sulphate reduction and possibly methane production also occurred within the sedimented organic matter in the bottom of this tank.

During the no-flow experiment 0.022 moles phosphate/d were produced in the sedimentation tank. With the ammonification rate of 0.2 moles N/d, this corresponds to an N/P ratio of ~ 9 for the organic matter which had decomposed. The measured N/P ratio of the organic matter which was drained from the sedimentation tank was only 4.5 (Table 2 and 3). It was estimated above that only $\sim 30\%$ of the organic matter which sediments in this tank was actually drained from the system. The remainder was decomposed. This suggests that the residual organic matter is richer in phosphorus than the organic matter which has been oxidized. This is the opposite of the situation typically found in natural systems where P is the more labile element (e.g. Krom and Berner, 1981). The food which was fed to the fish contained a significant amount of fish meal which contains bone material (Kissil and Lupatch, pers. comm.). Bone meal contains apatite which is not only indigestible to the fish but also resistant to bacterial decomposition. It is unknown at present whether this apatite and other resistant phosphorus phases are ultimately available to be recycled into the environment via microbial decay (Cowey and Cho, 1991).

C. Ulva tank:

The principal process which removed nutrients from the water column was uptake by seaweed. Most of the ammonia-N supplied to the Ulva tank was removed, often down to levels of less than 10 micromolar. Cohen and Neori (1991) have shown that with N fluxes of up to $6 \text{ g N m}^{-2} \text{ d}^{-1}$, the rate of ammonia-N uptake by the seaweed is dependant on the flux. The fluxes in the present system ranged from $0.3\text{-}3.5 \text{ g N m}^{-2} \text{ d}^{-1}$. Consequently, the biofilters had an important buffering capacity for ammonia-N levels in the system, with higher fluxes resulting in higher uptake rates. The seaweeds also removed significant amounts of phosphate from the water, though there was always excess phosphate present.

In our system, *Ulva lactuca* strongly preferred ammonia-N over oxidized N. Nitrite and nitrate were taken up by the seaweed when ammonia-N concentrations were below 1.2 micromolar, which happened only in the polishing tank receiving the system's outflow (Neori et al., in prep., see earlier in this report). The inhibition of nitrate uptake by seaweeds in the presence of ammonia-N is a well known process (Lobban et al., 1985). Only under very special conditions of nutrient concentrations and light did specimens of *Ulva rigida*, collected from the sea, show nitrate uptake in the presence of ammonia-N (Corzo and Niell, 1992). The seaweed N content, which was up to 6% of dry weight for the ammonia N-grown seaweed in the Ulva tank, was only up to 4% for the oxidized N-grown plants in the polishing tank (Neori et al., in prep.).

Table 4 compares the calculated uptake of ammonia-N in the Ulva tanks for each of the 24 hour sampling periods (corrected for an average nitrification rate of 0.15 Moles N/day) with the measured Ulva harvest. There was a close comparability confirming that the major process removing ammonia-N from the water column was uptake by Ulva. The computer simulation model estimates

the uptake of ammonia-N by *Ulva* using an equation based on Cohen and Neori (1991) and Neori et al., (1991). The predicted uptakes rates in the model are also similar to these values (Ellner et al., submitted, see later in this report) confirming that our quantitative understanding of this component of the system is valid.

D. Nitrification:

Total oxidized nitrogen (nitrite + nitrate) accumulated in the system due to bacterial nitrification. The conditions necessary for this process to occur, oxygen rich water with a significant quantity of ammonia-N, was present throughout the system except for the bottom of the sedimentation tank where oxygen was absent. From the 24 hour budgets it was calculated that the total amount of nitrification within the system was relatively constant throughout the year at about 10% of the total N input (Fig 3). At the beginning of the year most of the oxidized nitrogen was nitrite while as the year progressed the proportion of nitrite decreased relative to nitrate. The system was monitored for a further year (1993-4) and it was found that nitrate continued to be the principal oxidized nitrogen species exported (Neori et al., in prep., see earlier in this report, and data not shown). Thus the change from nitrite to nitrate was not related to temperature nor season. Various factors might have caused the observed nitrite accumulation in the system. Nitrite accumulation has been observed as a succession that occurs as the nitrification system develops with initial conversion of ammonia-N to nitrite and, after a certain lag period, conversion of nitrite to nitrate. This sequence is common in hypertrophic systems (Sharma and Ahlert, 1977). The lag period varies from a few days in freshwater systems (Shilo and Rimon, 1982) to a few weeks in marine systems (Manthe et al., 1984). However, in our system nitrite accumulated for several months. It is unlikely that this was caused by a simple succession of ammonia-N and nitrite oxidising bacteria. Low oxygen concentrations have been shown to cause nitrite accumulation by both nitrifiers (Helder and de Vries, 1983, van Rijn and Rivera, 1990) and denitrifiers (Tiedje, 1988). Zones of low oxygen were present in the system (sedimentation tank, bottom of fish and *Ulva* tanks). Laboratory incubation of samples from these zones resulted in nitrite accumulation (Dvir et al., unpubl.). The question as to the unusually slow disappearance of nitrite with the proceeding of the growth season remains open and deserves future attention.

Data from the no-flow experiment (Fig 4 and 6) estimated the net nitrification rate to be 0.15 Moles N/d in the fish tank and 0.05 Moles N/d in each of the three *Ulva* tanks. Preliminary observations (Dvir et al., unpublished) have suggested that nitrification occurs primarily on the walls of the *Ulva* tank, although some also occurred on suspended particles in the water and on the seaweed's surface. From the relationship between the total rates for seaweed growth and TOxN accumulation, it is clear that the nitrifiers could not outcompete the seaweed for the use of ammonia-N, probably because lack of colonization surface area. The area of the wet solid surfaces available for colonization by nitrifiers is likely to be an important practical determinant in the competition on ammonia-N between the seaweed and the bacteria.

E. Computer model results:

In a coinciding study to this, we developed a computer simulation model which was designed to represent a quantitative hypothesis about the processes controlling N cycling in this system, specified which processes were dominant, the rates of those processes, and the factors controlling those processes (Ellner et al., submitted, see later in this report). The model was

developed using the data and understandings presented in this paper. The match between the simulation model, and the experimental results were extremely good for a process based ecological model. It was able to describe the annual and diel cycles in the experimental system under normal operating conditions and most crucially to predict the consequences of changing the operating conditions (reducing the food supply by 50% and reducing the internal recycling rate of water by a factor of ~ 2.5). These latter tests involved independent data, that were not used in developing the model or estimating its parameters. Consequently, these results are a strong indication that we have identified the key processes determining the inorganic N balance in the system, and the factors that control those processes

F. Seasonality of the system:

The system had comparatively little seasonal variability in the principal outputs which have been monitored in this study (Table 2 and 3). This observation is a priori somewhat surprising since the temperature variation between winter and summer is greater than 10°C. It is known that several of the processes which occur in the system have Q10 values greater than 2-3 such as sulphate reduction and denitrification (Westrich and Berner 1988, Knowles 1982). The primary controlling factors in this system were the fish excretion rate and the Ulva uptake rate. Fish excretion is primarily controlled by the amount of food supplied and only to a much smaller degree by the temperature of the water (Lupatch, in prep.). Likewise the Ulva uptake was controlled primarily by the flux of ammonia-N through the Ulva tanks, except under very cloudy winter days which are unusual in Eilat (Neori et al., in prep., see earlier in this report). Nitrification rate, the next most important process, was apparently principally controlled by the amount of active surface area available for bacterial colonization and to a lesser extent by the concentration of ammonia-N flowing over that surface. Denitrification and sulphate reduction seem to be controlled principally by the amount of labile organic matter which is present in anoxic sites within the system. This, in turn, is controlled by the fish food input which was constant during this study. Sensitivity analysis of the simulation model indicates that changes in the rates of these processes (nitrification, denitrification, sulphate reduction) caused by temperature variations would induce far smaller percentage changes in water quality in the system (50-90% smaller for TOxN, 80-99% smaller for ammonia-N; Ellner et al., submitted, see later in this report). The resulting prediction, that TOxN levels should show greater seasonal variation than ammonia-N, is borne out by the experimental results (Ellner et al., submitted, see later in this report).

G. Pollution output from the system:

The Fishpond-seaweed system was designed to control water quality in the fish tank and to reduce the pollutant (nutrient) output into the environment. Because the total amount of fish produced by various mariculture system per kg of food fed is comparable, the percentages exported from various mariculture systems can be simply compared (Fig 7). Hall et al., (1992) found that 72% of the nutrients supplied to fish in seacages was lost to the environment, principally in dissolved forms. These nutrients are in part simply diluted and exported from the region of the cages or accumulated as organic rich deposits beneath the cages. The impact of such systems on the environment is generally only monitored by the extent of this organic rich deposit. Other effects such as changes in the local ecology are more difficult to quantify and are often ignored.

The quantity of nutrients exported from the fishpond-phytoplankton system is similar to that lost from the seacages (71-74%). However because the plankton can be used for bivalve culture

there is a possibility of growing a second crop which will remove part of the particulate matter and also provide an additional commercial return (Shpigel et al., 1993). The problem is that the uncontrollable bloom and crash cycle as well as the type of phytoplankton which grow makes it problematic to grow a bivalve crop. The fishpond-seaweed system only loses 20-27% of the nitrogen input to the environment (31% if the deficit in N is not included). There was an additional crop of sea lettuce (*Ulva lactuca*) which is of commercial value. Sea lettuce is eaten directly as human food, particularly in the Far East. It also can be used to cultivate marine invertebrate macroalgivores such as abalone, which are prized food in many parts of the world.

While the output stream contained less nutrients than other comparable intensive mariculture systems, it still contained more dissolved and particulate nutrients especially phosphate, than is considered desirable. In this pilot plant we used a small (600l) tank with seaweed as a polishing stage. While this did result in a further reduction of the nutrient load (31% $\rightarrow$ 26%) in the effluent, it was not very efficient. The ammonia-N level in the stream to the polishing tank was low with most of the N in the form of oxidised nitrogen. Since seaweed incorporate nitrate less efficiently than ammonia, neither nitrate nor phosphate were completely removed from the outflow. Now that the characteristics of the effluent stream are known more precisely, it would be possible to design a polishing stage specifically to further reduce the load of nitrate, particulate N and phosphate to the levels necessary to avoid approaching the accommodative capacity of the receiving environment.

VII. CITED LITERATURE

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FINAL REPORT

Chapter IV: SIMULATION MODEL OF RECIRCULATING MARICULTURE SYSTEM WITH SEAWEED BIOFILTER:
 DEVELOPMENT AND EXPERIMENTAL TESTS OF THE MODEL

Introduction

Simulation modeling is a well-established tool for studying and managing biological systems, including aquaculture systems (e.g. Clark 1985, Walters 1986), and models have been developed for a number of cultured species (see e.g. reviews by Allen et al. 1984, Leung 1986, Bolte et al. 1986, and in Lannan et al., 1986). The purposes served by such models can be divided into three broad categories:

1. Scientific understanding of the system. In the initial stages of a study, the process of building a model organizes what is already known, and identifies areas of ignorance. A completed simulation model represents a quantitative hypothesis about the processes occurring in the system, that specifies which processes are dominant, the rates of processes, and the factors controlling those processes. Comparisons between model predictions, and the outcome of experiments on the system, indicate how well the hypotheses in the model correspond to the actual system. If model predictions are not confirmed, the model is an arena for developing new hypotheses and determining which are consistent with the available information.

2. System design. Simulation experiments are a cheap and rapid procedure for exploring many possible choices for system design (e.g., volumes, stocking intensity) and operating procedures (e.g., water inflow and circulation rates), so that experimental work can utilize only those that appear most promising.

3. Forecasting and management. A working model can be used by managers to predict the effects of alternative management decisions (e.g., how to respond to a decline in water quality), and to optimize ongoing adjustments of the system in response to changing conditions.

Here, we report on a simulation model of N dynamics in an experimental recirculating mariculture system which has been constructed and operated in Eilat, Israel. The system, described by Neori et al. (1993), cultures gilthead seabream (*Sparus aurata*) and uses a seaweed biofilter to maintain water quality and reduce nutrient export. We developed the model for the first two of the purposes listed above: (1) To test our hypotheses about the magnitude, and location, of the input, output, and transformation processes affecting N in the system. (2) To propose and compare possible designs for scaling up from the "pilot" experimental system to a commercial scale system. In addition, the current model can provide a basis for a modeling full scale commercial systems.

In this paper we focus on the model and its contributions to our understanding of the system. We outline the assumptions of the model, the scientific basis for our assumptions, and the data and methods used to estimate model parameters.

The model is based on a small number of "key" processes which we believe are dominant in the N dynamics, and therefore it is structurally quite simple. Nonetheless, we find that it is able to duplicate accurately the behavior of the system in all of the experiments which were used for building the model and estimating parameters. We have also tested predictions of the model against independent data -- experiments which were not used for estimating any model parameters -- and find good agreement. These results suggest that the model provides a good mechanistic description of the processes responsible for N dynamics in the system.

In two companion papers, we turn to issues of scaling up to a commercial system. Simulation experiments, with parameters corresponding to larger systems, are used to suggest the potential, and limitations, of recirculating mariculture systems with seaweed biofilters. The first paper builds on the model described here, to determine conditions under which acceptable water quality can be maintained (Ellner et al. 1994a). For those calculations we assume that, as in our experimental system, the seaweed biofilter is able to prevent algal blooms. In the second companion paper, we examine the validity of this assumption, by modeling algal population dynamics in a recirculating mariculture system.

The Experimental System

Description. The experimental system consisted of four fiberglass tanks (2.5 m³; 3.3 m² each), one for fish culture containing 1790 l of sea water and the other three for seaweed culture containing a total of 6390 l (Figure 1). Water was circulated by an electric pump from the fish tank via a small (400 l) sedimentation tank, to the three seaweed (ulva) tanks arranged in parallel. Water then returned to the fish tank via gravity. The seaweed was kept in the seaweed tank by screens at each end of the tank. The whole system contained 9200 l of sea water which was circulated internally at a rate of 800-1000 l/hr. Each day, 2000 l of fresh sea water was pumped into the system in two four-hour pulses of 1000 l each beginning at 08:00 and at 16:00. There was a simultaneous outflow of 1800 l (input less evaporation and sampling; Neori et al, in prep.) which flowed out to the sea via a 600 l "polishing" tank. A small volume of water with accumulated sediment was drained daily from the sedimentation tank.

Beginning in 1991, the fish tank was stocked *Sparus aurata*. The fish were fed daily (08:30) 600g of the fish diet described in Krom and Neori (1989). Fish numbers and biomass were adjusted so that a feeding ration of 600g could be maintained throughout the study period. The fish tank was covered to limit photosynthetic activity and was aerated via holes in the tank base. The seaweed tanks were stocked with *Ulva lactuca* at an initial stocking

FIGURE 1: See Fig. 1 in Chapters I and III
Diagrams showing the system and water flows among the
compartments.

density of 3 kg. The seaweed was harvested weekly and immediately restocked at the original density. The experimental system was run in this mode until 1993. Water quality was monitored throughout the sampling period on a regular basis (Neori et al., in prep.).

24 Hour experiments. A series of six intensive 24 hour samplings for total nutrient budgets were carried out between October 1991 and May 1992. The sampling began at 08:30 and continued every 3 or 4 hours until 08:30 the following morning. Water samples were taken from the fish tank, the sedimentation tank, each of the three seaweed tanks and the polishing tank. The fresh sea water inflow was sampled once per sampling period in the morning. One liter of water was collected for each sampling for dissolved and particulate matter measurement. In addition the sump of the sedimentation tank was drained for several seconds and the entire water sample (with its sediment) was collected. The chemical analytic methods are described by Krom et al. (in prep.).

Additional experiments. Three experiments were conducted under operating conditions different from those maintained throughout the 24 hour experiments. In February 1993, a "low flow" experiment was conducted in which the recirculation rate of water between the fish and seaweed tanks was reduced from 20000 to 8450 l/d. In May 1993 a "no flow" experiment was run in which the flow of water in the system (input and recirculation) was entirely stopped for 5.25 hours (14:00 -> 19:15). In September 1993, a "low food" experiment was conducted in which the feeding rate was halved for 3 days. Sampling and chemical analyses were the same as in the 24 hour experiments, except that in the "no flow" experiment samples were taken at 14:00 and then hourly from 5:15 until 19:15.

Outline of the model

In this section we outline the structure and biological assumptions of the model. An annotated listing of the model (FORTRAN source code) is available on request from the first author (e-mail to ellner@stat.ncsu.edu).

Our model is a process-level compartmental model which describes the changes over time in the amounts of Ammonia ($\text{NH}_4 + \text{NH}_3$) and Total oxidized Nitrogen ($\text{TOxN} = \text{NO}_2 + \text{NO}_3$) in each of several *compartments* (components parts of the system). The compartments in our model are

1. Fish tank

2. Sedimentation tank
3. Seaweed tanks
4. Polishing tank

In the actual experimental system there are 3 (or occasionally 2) separate seaweed tanks, but we treat all tanks as a single unit in the model. Thus the model has a total of 8 state variables (amounts of the two N species in each of the four compartments). Phytoplankton are assumed to be at negligible densities, as occurred in the experiments; hence the model cannot be applied in circumstances that lead to plankton blooms.

The equations defining a compartment model take the form

$$(1) \quad \begin{aligned} &\text{Rate of change of amount in compartment } i = \\ &\quad \text{Internal production rate} + \text{Rate of input} \\ &\quad - (\text{Internal consumption rate} + \text{Rate of outflow}) \end{aligned}$$

The amounts of ammonia and TOxN in the inflowing sea water are negligibly small compared to amounts in the system, and are omitted from the model, so that all "input" terms in equation (1) are inputs from other compartments in the system. However "outflow" includes all losses to the external world.

We assume that each compartment is well-mixed, so that transfers between compartments (inflows and outflows) are described by standard mass-balance equations. For example, the rate (in moles/hr) at which TOxN flows out from the fish tank is given by the product

$$(2) \quad [TOxN] \text{ in fish tank (moles/l)} \times \text{Water outflow rate from fish tank (l/hr)}.$$

Similar equations apply to all inter-compartment transfers, and all losses to the external world (leakage, and outflow from the polishing tank).

Thus it is really only necessary to model the production and consumption processes within compartments. The process included in the model are summarized in Table 1.

Ammonia excretion. The main source of ammonia is excretion by the fish. Based on Porter et al. (1987) we assumed that 1/3 of all N consumed is excreted as ammonia. In addition, based on the DON (dissolved organic nitrogen) budget for the experimental system, we assume that 20% of the N consumed is excreted as DON but quickly converted

Table 1. Processes included in the simulation model, and their rate equations. All concentrations are in units of μM , and all rates are given in units of moles/d. $[A]$ = concentration of Ammonia-N, $[\text{TOxN}]$ = concentration of $\text{NO}_2 + \text{NO}_3$ (total oxidized N).

<u>Process</u>	<u>Location</u>	<u>Rate</u>
Ammonia excretion by fish	Fish tank	Feeding rate (moles N/d) x equation (1) in text
Ammonia production by sulfate reduction	Sedimentation tank	$0.103 \times$ supply rate of N in fish food (moles N/d)
Ammonia uptake by <i>Ulva</i>	Seaweed tanks	See text, section <i>Ammonia uptake by Ulva</i>
Nitrification of ammonia	Fish tank	$.003[A]$
Nitrification of ammonia	Seaweed tank	$.011[A]$
Denitrification of oxidized N	Sedimentation tank	$.0008735[\text{TOxN}]$
Leakage	Fish and seaweed tanks	$.005/\text{d}$
Evaporation	Fish and seaweed tanks	$.001$ pond temperature (C)

to ammonia within the fish tank. Hence 53% of the N consumed each day is excreted as ammonia. The excretion rate varies over the day, and the data of Porter et al. (1987) were used to estimate an equation describing this trend:

$$(3) \quad E(t) = \begin{array}{ll} 2.314 - 3.5t & \text{for } 0 < t < 12 \text{ hrs} \\ 0.561 & \text{for } t > 12 \text{ hrs} \end{array}$$

$E(t)$ gives the instantaneous excretion rate (moles N/d) t hours after the last feeding, per mole of N ingested. The fish in the experiments of Porter et al. (1987) were given a single feeding, so the excretion rate model should not be applied to fish that receive more than one feeding per day.

Sulfate reduction. The other ammonia source included in the model is anoxic sulfate reduction in the sedimentation tank. This was assumed to be proportional to the POM production rate by the fish, which is in turn proportional to the feeding rate (moles N/d). A production rate of 0.206 moles ammonia N/d (from the "no flow" experiment, Krom et al. in prep), divided by the estimated feeding rate of 2 moles N/d, gives the proportionality constant 0.106.

Ammonia uptake by Ulva. These rate equations are based on the experiments of Cohen & Neori (1991), scaled up on a surface area basis. That is, each m^2 of Ulva tank surface area is treated as equivalent to one experimental tank ($0.6m^3$, $1m^2$) from Cohen & Neori (1991). Ulva uptake is assumed to be limited by ammonia concentration and light, including both seasonal and diel variations in light intensity.

(a) The "winter" experiments (Nov-Feb) of Cohen & Neori (1991) were used to estimate parameters for a Michaelis-Menten equation relating the uptake rate in daylight to ammonia concentration. Parameter estimates are $V_{max}=0.1833$ moles/d, $K_s=4.09$ μM , obtained by nonlinear least squares using the data of Cohen & Neori (1991). Thus the effect of ammonia concentration on uptake rate is given by

$$(4) \quad f_1(C)=0.1833[A]/(4.09 + [A]),$$

for $[A]$ =ammonia-N concentration in μM , f_1 = ammonia uptake rate in moles/d.

(b) To model the higher uptake rates which occur during spring and summer, uptake rates from Cohen and Neori's "summer" (May) experiments were compared with the rate predicted by the "winter" model. The summer uptake rates were higher than the predictions by 37.6% on average. This is nearly identical to the change in average light intensity (photons/sec) between the "winter" and "summer" experimental periods. We therefore modeled the seasonal trend in uptake rates as being proportional to the trend in light intensity. The resulting equation, using irradiance measurements provided by the Elat Meteorological Station, is

$$(5) \quad f_2(d) = 1.249 + 0.01029 \sin(2d/365) - 0.3013 \cos(2d/365)$$

where d is the Julian date. The product $f_1(C)f_2(d)$ is then taken to be the uptake rate at ammonia concentration C , on day d , at 8am Israel Standard Time (when the daytime measurements of Cohen & Neori (1991) were taken).

(c) Uptake rate during the night was estimated from the data of Cohen & Neori (1991). We found no consistent pattern of change with time over the night, so the data were used to model the average uptake rate over the night as a function of the ammonia concentration in the Ulva tanks during the previous afternoon, which we interpret as a surrogate for the nutrient status of the Ulva. The model is

$$(6) \quad U_{\text{night}} / U_{\text{day}} = 0.9 \exp(-0.06 C_{15})$$

where U_{day} is the uptake rate predicted by the Michaelis-Menten daylight uptake model, and C_{15} is the ammonia concentration (μM) at 3pm the previous day. The rate U_{night} is assumed to hold from sunset until the next sunrise.

(d) To model the diel variation in uptake rate during daylight hours, a sine-wave was used to interpolate between the nighttime uptake rate (assumed to hold from sunset until the following sunrise) and the daytime uptake rate function (assumed to hold at 8am IST).

Nitrification of Ammonia. This is the only source of TOxN in the model. Nitrification was assumed to follow first-order kinetics (i.e., production rate proportional to ammonia concentration). The rate constant was estimated as the ratio between the production rate obtained in the "no-flow" experiment, and the average concentration over all nutrient budget experiments. Separate rate constants were calculated for the fish and ulva tanks; nitrification elsewhere in the system was assumed to be negligible.

Denitrification of TOxN . This was assumed to occur only in the sedimentation tank, and to follow first-order kinetics. The rate constant was calculated by the same procedure as was used for nitrification.

Results

When it is run with parameters corresponding to experimental conditions, the model reproduces very closely the experimental results. The first set of comparisons involves the data from the 24hr experiments described above.

Figure 2 compares the experimental data with the model's predictions for seasonal and daily fluctuations in Ammonia. The model slightly under-estimates the daily maximum in the fish tank, but otherwise there is close correspondence. Figure 3 makes the same comparison for TOxN . Here the goodness of fit is more difficult to evaluate because of the

Figure 2. Comparison between the simulation model and results from the 24 hour intensive sampling experiments. The curves are the minimum and maximum daily values of ammonia-N in the fish tank (square) and seaweed tank (triangle). A: Experimental results. B: Model output. The output of the simulation model was "sampled" at the times used in the experiments so that the results would be directly comparable.

Figure 3. As in Figure 2, for TOxN.

Figure 4. Comparison between the simulation model and results from the "low flow" experiment. The curves show the values of ammonia-N in the fish tank(square), seaweed tank (triangle), and sedimentation tank (x) over the sampling period. A: Experimental results. B: Model output.

Figure 5. As in Figure 4, for TOxN.

Figure 6. Comparison between the simulation model and results from the "low food" experiment. The curves show the values of ammonia-N in the fish tank(square) and seaweed tank (triangle) over the sampling period. A: Experimental results. B: Model output.

Table 2. Sensitivity of model output to changes in parameter values. Sensitivities were calculated by running the model with parameters changed $\pm 5\%$ from their nominal values and calculating the % change in four measures of response: the maximum concentrations of Ammonia-N and of TOxN recorded in the fish tank, in July and December. A sensitivity value of 1 indicates that a given change in parameters causes an equal % change in the measure of response; a sensitivity of 0.2 indicates that the % change in the measure of response is only 1/5 as large as the % change in parameter value. Sensitivities below 0.01 are indicated by --.

	July		December	
	Ammonia-N	TOxN	Ammonia-N	TOxN
Fish tank volume	-0.15	0.04	-0.12	-0.02
Sediment tank volume	--	0.02	--	--
Ulva tank volume	--	-0.02	0.03	-0.02
Water inflow rate	-0.01	-0.72	-0.04	-0.73
Recirculation rate	-0.67	-0.35	-0.58	-0.09
Ulva uptake/m ²	-0.13	-1.3	-0.35	-1.5
Feeding rate	1.1	2.1	1.3	2.4
[Ammonia N] in inflow	--	--	--	--
Leakage rate	--	0.02	--	0.02
Nitrif. rate, Fish tank	-0.13	0.29	-0.18	0.09
Nitrif. rate, Ulva tank	-0.01	0.26	-0.09	0.15
Denitrif. rate, Sed-tank	--	-0.34	--	-0.32
Ammonif. rate, Sed-tank	0.02	0.27	0.08	0.36

greater variability in the data, and the apparent non-seasonal trend in the experimental system. However, there is a good match to the magnitudes of seasonal and diel variation, and to the relative magnitudes of diel variation in the fish and seaweed tanks.

It should be emphasized that we did not make any attempt to "calibrate" the model to these data (i.e., adjust parameters to maximize the goodness of fit). Rather, subsets of the data were simply used (as described above) to estimate parameters for individual rate equations. Still, the comparisons in Figures 2 and 3 suffer from the fact that the 24 hour experiment data were used in formulating the model and estimating parameters. In particular, the choice of functional forms in the rate equations for Ammonia uptake by *Ulva*, and for denitrification, were guided by these experimental data and alternative models that resulted in large departures from the data were discarded.

To allow a more stringent evaluation of the model, the "low food" and "low flow" experiment data sets were held in reserve. These data sets are separate from those used to develop the model, and moreover they involve different operating conditions. For the "low flow" experiment, the model results for ammonia-N (Figure 4) are on-target for the fish tank and seaweed tank, predicting very well the rate and magnitude of the changes that occur when the recirculation rate is drastically reduced. However, the model failed to predict the rapid increase in ammonia-N in the sedimentation tank. For TOxN (Figure 5), the model correctly predicted that there would not be large changes in concentration, but predicted larger fluctuations than were actually observed in the fish tank or sedimentation tank.

The "low food" experiment was conducted shortly after the system had been completely cleaned, and all bacteria killed. The nitrification rate was consequently far below normal, resulting in uniformly low TOxN levels ($<10\mu\text{M}$) throughout the experiment. We therefore reduced the rate constants for nitrification in the model by 95%, to match the observed levels of TOxN. Figure 6 compares observed and predicted levels of ammonia-N during the low food experiment. The only significant difference is the peak of ammonia-N in the fishpond during days 1 and 5, when the fish were fed at their normal rate. Peaks of roughly $90\mu\text{M}$ are predicted for days 1 and 5, vs. observed peaks of about $70\mu\text{M}$ on day 1 and $60\mu\text{M}$ on day 5.

Sensitivity analysis of the model (Table 2) indicates that the levels of Ammonia-N and TOxN in the system are not extremely sensitive to any of the parameters. Sensitivity analysis ranks parameters according to the relative size of their effects on model output. The sensitivity of the model to parameter p_i is defined to be the ratio (% change in model output)/(% change in parameter value), evaluated by running the model before and after a small change in the parameter (here +5% and -5% changes were used). Table 2

summarizes the results for four measures of model output: the peak values of Ammonia-N and TOxN in the fish tank during July and December.

The highest sensitivity parameters are those having to do with Ammonia-N supply by the fish, and Ammonia-N uptake by the seaweed, which reflects the dominant role of these processes in the N budget of the system. These are followed by the parameters having to do with the inflow of fresh sea water, and the recirculation rate between the fish and seaweed tanks. The inflow rate has little effect on Ammonia-N, presumably because water recirculated from the seaweed tanks is not much higher in Ammonia-N than is fresh sea water; however the recirculated water has not been stripped of TOxN, so additional fresh inflow has a large effect on TOxN levels in the fish tank. The sensitivity to recirculation rate is surprising: the daily recirculation rate is already quite high (roughly 3 times the total system volume), so we expected that further increases would have little impact.

Sensitivities to the internal transformation rates (the last four items in Table 1) were all well below 1, indicating a certain amount of homeostatic response to variation in these rates, but also well above 0. The fact that these sensitivities are not near 0 is important for interpreting the model's success at matching the experimental data, since it indicates that any substantial errors in the modeling of these processes would be manifested in the model output, and would generate mis-matches between the model and the data.

Discussion

The match between the simulation model, and the experimental results, is extremely good for a process-based ecological model. The model was able to describe the annual and diel cycles in the experimental system under normal operating conditions, and to predict the consequences of changing the operating conditions (the "low food" and "low flow" experiments). These latter test involved independent data, that were not used in developing the model or estimating its parameters. Consequently, these results are a strong indication that we have correctly identified the key processes determining inorganic N balance in the system, and the factors that control those processes. Thus, the model has been successful at the first of the goals set out in the Introduction.

The second goal -- system design -- is more difficult to accomplish, because it requires extrapolation far beyond the range of conditions in the experiments used to construct and test the model. For example, nitrification rate is modeled as a first-order process,

$$\text{Nitrification rate} = C \times \text{Ammonia-N concentration},$$

where C is a constant whose value was estimated from data. This rate equation appears to be a reasonably good approximation for the experimental system, and it would therefore be reasonable to apply the same equation in modeling a scaled-up system. However, at this point we do not have any experimental data that can be used to determine the value of C for a differently designed system, with different tank volumes, tank surface areas, flow rates, etc.

However, it is possible to explore at least tentatively the potential for mariculture in a scaled-up system, by doing a hypothetical scale-up in which we increase the feeding rate in the model (corresponding to a higher stocking density of fish) and maintain water quality by increasing the number of seaweed tanks. The results of this simulation experiment are summarized in Table 3. It appears from these results that the experimental system could withstand a much higher stocking density of fish, without generating dangerously high levels of ammonia-N in the fish tank. Adding seaweed tanks reduces the ammonia peak, of course, but there is a large "diminishing returns" to adding seaweed tanks: the marginal gain from each additional seaweed tanks drops with each added tank.

In summary, we have developed a simulation model for a recirculating mariculture system with seaweed biofilter, tested the model against experimental data on the system, and indicated how the model can be used to help guide the process of scaling up to a commercial system. The results in Table 3 would only be one ingredient in planning for the effective use of this technology. More extensive explorations of the model's predictions for larger scale systems, including considerations of the effluent burden, will be the focus of a sequel to this paper.

Table 3. Simulation results for a scaled-up version of the experimental system, with increased feeding rate (i.e., increased stocking density of fish) and additional seaweed tanks. The table entries are the peak levels of ammonia-N in the fish tank (μM) during December and July. The top left-hand corner of each table (feeding rate 2 moles N/d, 3 seaweed tanks) is the actual configuration of the experimental system.

December

Feeding rate (moles N/d)	Number of Seaweed Tanks			
	3	5	7	9
2	93	84	83	83
4	176	149	114	100
6	255	225	195	167
8	343	297	264	235
10	435	370	339	306

July

Feeding rate (moles N/d)	Number of Seaweed Tanks			
	3	5	7	9
2	85	83	83	82
4	161	118	99	98
6	238	200	161	112
8	325	271	230	192
10	416	343	298	256

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