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# An artificial productive ecosystem based on a fish/bacteria/plant association. 2. Performance

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## Abstract

An artificial ecosystem was developed with the aim of associating fish production with a vegetable crop purifying the fish water, in a closed system. The nitrogenous compounds excreted in dissolved form by the fish, and transformed by the bacteria, provide nitrogen for the plants. Previous studies have focused on the system's design and the management of the plant compartment in order to achieve a state of equilibrium.

The present paper deals with the performance of the ecosystem over a 2 year period in different conditions of fish and plant culture. Yield measurement of the biological production and the dynamics of the nitrogen transfers in the ecosystem made it possible to define for each trial the efficiency of the system both in terms of productivity and of waste nitrogen recovery.

In the spring–summer trial, 80 tomatoes in staggered crops were associated with 216 growing fingerlings. Fish growth paralleled that obtained in a traditional breeding tank and tomato yield reached 70% of the conventional hydroponic culture but with a drastic saving in fertilizers. The recirculating water was well purified as its nitrogen content remained low at 1 mmol $l^{-1}$. About 60 % of the nitrogen supplied was recovered from fish (31%) and plants (28%).

In the winter trial, 176 lettuce cultivars were associated with 65 adult fish. The plant production varied with the lettuce genotype used, with only butterhead lettuces reaching the same yield as in conventional hydroponics. The size of the plant compartment was too small in relation to the fish compartment so only 29 % of the nitrogen supplied was recovered (fish 21 %, plants 7.5 %). Therefore, the water nitrogen content increased to 15% of the nitrogen supplied.

When the main conditions to ensure equilibrium of the ecosystem are satisfactory, size relationship between the three interacting compartments, dynamic management of the fish compartment (growing fingerlings) and the plant compartment (staggered crops, application of a mineral complement), biological production can reach a high level with low fresh water input and low pollution of the environment. The nitrogen balance highlighted the great efficiency of the purifying plant compartment (nitrogen assimilation by plants was high and nitrogen remained low in water) which has been not observed in earlier experiments with artificial ecosystems.

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## 1. Introduction

In a preceding paper (Quilleré et al., 1993) we have described an artificial ecosystem associating fish (tilapias), bacteria and plants (tomatoes) and defined the required conditions for its equilibrium. The first results relative to its performance were obtained in suboptimal conditions. More significant results were obtained during a 2 year experimental period, in varied conditions of production, more or less favourable to equilibrium in the ecosystem.

The present paper deals with two contrasted periods of functioning: (1) a spring–summer period (February–July 1989) with fish production from fingerlings and vegetable production by staggered tomato crops; (2) a winter period (December–February 1990) set aside for a trial of plant diversification with a lettuce crop and adult fish. For each period the biological productions and the components of the nitrogen balance of the ecosystem were quantified and discussed in relation to the conditions of the ecosystem performance.

## 2. Materials and methods

The system with its three compartments (fish, bacteria, plants) has been already described (Quilleré et al., 1993). For the new experimental periods of spring–summer and winter, Day 0 was 31 January, day of complete draining and cleaning of the system.

### 2.1. Animal compartment (Fig. 1)

In order to optimize fish growth during the spring–summer experiment, fingerlings (mixed population) hatched on 1 October were introduced stepwise in the system already containing adults. Six days before Day 0, 100 fingerlings (average weight 14 g) were introduced in the fish tank but isolated in a latticed casing. Then, 116 fingerlings (average weight 21 g) were added on 30 March (Day 58). In order to maintain the nitrogen supply to the plants during the beginning of the experiment, the 69 adult fish already present were discarded only on 22 May (Day 111). On 20 June (Day 140) 93 fish died for an unidentified cause, perhaps a fleeting peak of $NO_2$ resulting from the denitrification of feeding residues accumulated at the bottom of the fish tank.

Fish were fed daily (one to three times a day) with a commercial carp feed (92 % dry matter, 5.6 % N). During the spring–summer trial the daily ration of the 69 adult fish varied between 200 and 250 g (Fig. 1). For fingerlings it was readjusted after every weight testing and increased from 50 to 650 g. From 30 March (Day 58) to 19 June (Day 139) the nitrogen flow from the animal compartment regularly increased while the feeding rate was maintained at 1.8–1.9 % of the body weight of the growing fish.

The winter experiment began on 22 December (Day 325) with a complete change of water in the fish tank. The daily ration of the 65 fish was maintained at 300 g $day^{-1}$ because no attempt was made to improve animal production as the fish were now adult; as a result N supplied by the animal compartment was almost constant throughout this period.

### 2.2. Bacterial compartment

Compared with the initial pilot system (Quilleré et al., 1993) the volume of the bacterial filter was increased from 50 to 150 kg of granular clay BIOGROG.

### 2.3. Plant compartment (Fig. 1)

For the spring–summer trial 80 tomato plants, var. Ferline, were set up on 6 February (Day 6), at the stage of the first floral cluster. The mineral complement supply (Quilleré et al., 1993) started 4 days later. Half the plants were topped above the first floral cluster on 28 February (Day 28) and the remainder topped after the tenth floral cluster on 16 May (Day 105). Simultaneous

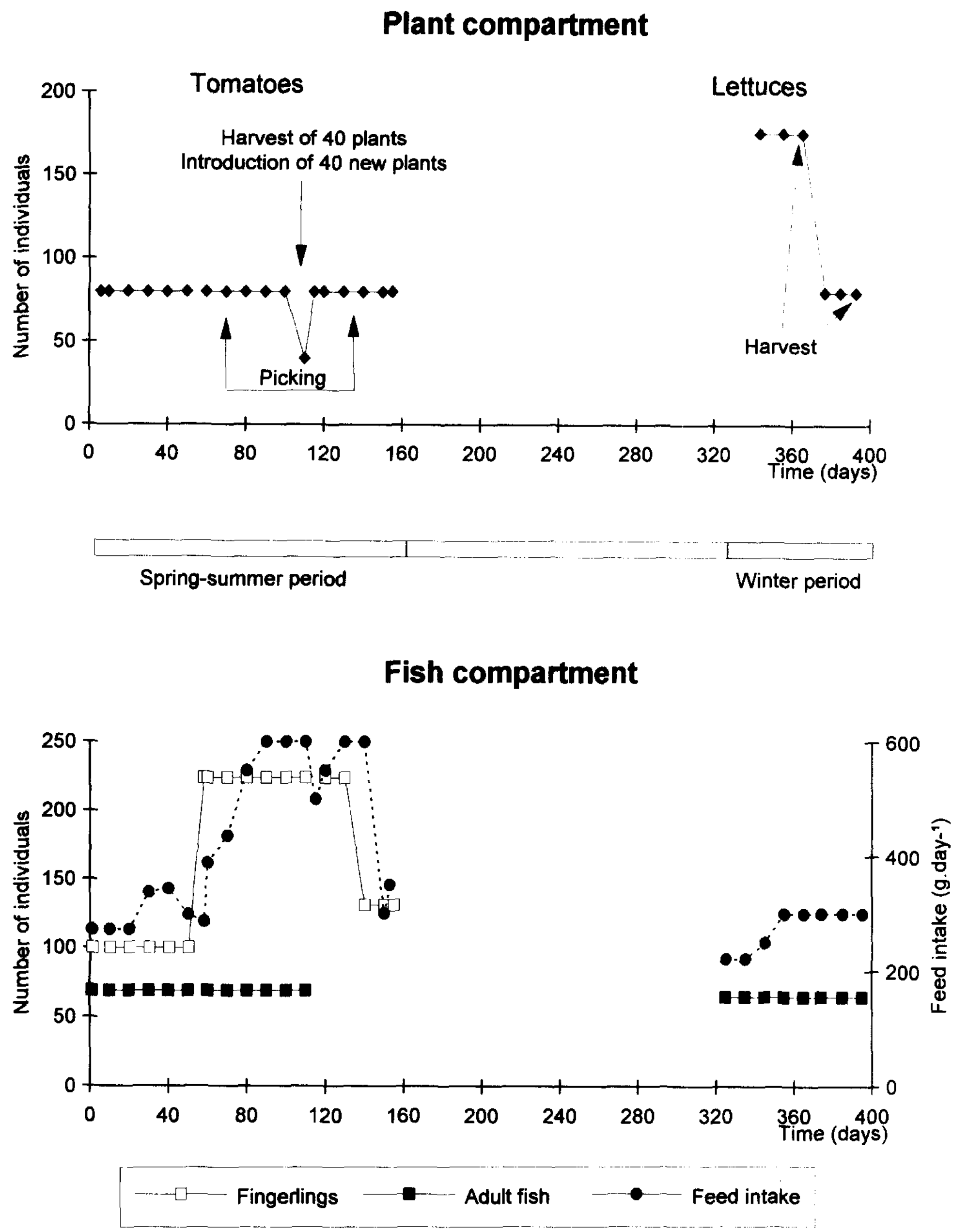


Fig. 1. Evolution of plant and fish populations and feed intake during the 2 experimental periods of spring-summer and winter.

crops of tomato plants at different phenological stages were set up to maintain a continuous nitrogen sink to counterbalance the nitrogen animal source. Fruit picking extended from 20 April (Day 79) to 18 May (Day 107) for the one-cluster plants and from 3 May (Day 92) to 3 July (Day 153) for the ten-cluster plants. On 25 May (Day 114) the one-cluster plants were replaced by a set of new plants.

For the winter trial, 176 3 week old lettuce plants were set up on 9 January (Day 343) and the mineral complement supplied 9 days later.

The genotypes grown represented the typical lettuce types for winter culture in unheated greenhouses or field spring–summer culture (butterhead, crisphead, loose-leaf and cos lettuce). As the temperature of the circulating water was 28°C, the risk of rapid bolting was tentatively counterbalanced by a 16 h extra lighting period for half the plants. As plant density progressively became critical for normal individual growth, on 12 February (Day 377) one plant out of two was discarded, thus leaving 80 plants. Final cropping took place on 28 February (Day 393). A conventional hydroponic crop was grown at the same time, without extra lighting but with a nutrient solution (Lesaint and Coïc, 1983) maintained at 28°C.

### 2.4. Measurement of biological parameters

Physico–chemical characteristics of the recirculating water (pH, conductivity and nitrogen compound contents) were measured three times a week as already described (Quilleré et al., 1993). Water input in the system and the mineral complement supplied to plants were noted.

The growth rate of fish was measured by frequent individual weighing under anaesthesia (phenoxy 2 ethanol, 0.3 ml $l^{-1}$).

At the end of each experiment dry matter and nitrogen content (by Kjeldahl method) were determined in plant material and fish.

## 3. Results and discussion

### 3.1. Biological production

The production levels of the fish and the plant compartments are presented in Table 1. The results obtained in the first experimental period (Quilleré et al., 1993) are reported as '1° period (autumn)' in Tables 1, 3, 4 and 5.

#### 3.1.1. Animal growth

In the spring–summer period, the average individual weight of the fingerlings increased in an exponential manner (Fig. 2) from 13.8 g$\pm$5 (6 days before Day 0) to 224.6 g$\pm$81 (Day 178). Standard deviations are large because of the sexual dimorphism expressed during tilapia growth (Mélard and Philippart, 1981). Mélard (1986) has devised a predictive model for the mean weight increase of fish, for a mixed population, under optimal breeding conditions. Experimental results fit satisfactorily with calculations based on Mélard's model: the weight increase of fish from 20 to 100 g amounted to 90 % of the calculated values and from 100 to 200 g, 98 % of the calculated values.

The feed conversion ratio (grams of feed per gram of fish weight gain) estimated on fingerlings varied from 1.25 to 1.61 according to the initial fish weight. It agreed with results obtained in non recirculating systems (Viola and Arieli, 1982; El Sayed, 1990). For adults it ranged from 1.9 (autumn) to 3 (spring–summer).

Thus, in this ecosystem, fish growth was satisfactory with regard to the results obtained in intensive breeding systems without recirculating water.

#### 3.1.2. Plant growth

Fruit yield of the tomato crop was 0.5 kg per plant in the one-cluster plants and 5 kg per plant in the ten-cluster plants. This average yield of 0.5 kg per plant per cluster represents 70 to 85 % of the cluster yield of the Ferline variety in intensive field culture (Odet, 1987; Odet and Jay, 1987). Tomato production in the ecosystem was about 60–75 % of the production of Dutch cultivars in a greenhouse, for an equivalent period (Letard and Le Quillec, 1990; Boidin et al., 1993).

Compared with other kinds of recycled systems, fruit yield was on the same level: 0.5–3.7 kg per plant with *Tilapia aurea* (Watten and Busch, 1984); 4.6 kg per plant with Catfish (Lewis et al., 1978) or 1.9–8.9 kg per plant depending on variety or year (Lewis et al., 1981); or up to 9 kg per plant (Sutton and Lewis, 1982).

Lettuce production amounted to 21 kg of edible matter (heads, fresh weight) for 80 plants. Results were highly variable among the tested genotypes: from 100 to 325 g without extra lighting (Table 2), to 460 g with extra lighting. Depending on cultivars, the yield represented 60 to

Table 1
Simultaneous fish and plant productions in the ecosystem during three experimental periods

| | | Number of individuals | Duration (days) | Production (kg) |
|---|---|---|---|---|
| *1° period (autumn)* | | | | |
| Fish | Adults | 69 | 102 | 5.85 |
| Plant | Tomatoes (five clusters) | 60 | 91 | 45 (fruits, F.W.) |
| Non-edible plant matter: 6.5 kg D.W. | | | | |
| *2° period (spring–summer)* | | | | |
| Fish | Adults | 69 | 111 | 6.4 |
| | Fingerlings[a] | 100 | 159 | 34.2 |
| | | 116 | 95 | |
| Plant | Tomatoes (ten clusters) | 40 | 147 | 201 (fruits, F.W.) |
| | Tomatoes (one cluster) | 40 | 108 | 20 (fruits, F.W.) |
| | Tomatoes[b] (vegetative growth) | 40 | 39 | |
| Total non-edible plant matter: 27.4 kg D.W. | | | | |
| *3° period (winter)* | | | | |
| Fish | Adults[c] | 65 | 63 | 7.4 |
| Plant | Lettuces[d] | 80 | 50 | 21 (heads, F.W.) |
| | | 96 | 34 | |
| Total non-edible plant matter: 0.6 kg D.W. | | | | |

[a] Ninety-three fish died 13 days before the end of the period but the final production includes their weight.
[b] The 40 initial one cluster plants were replaced by 40 new plants whose growth was only vegetative. They are included in the total non-edible matter of the period.
[c] The fish weight increase was only measured after 94 days and re-calculated for the 63 days of the period.
[d] The 96 lettuces which were discarded early are included in the total non-edible matter of the period.

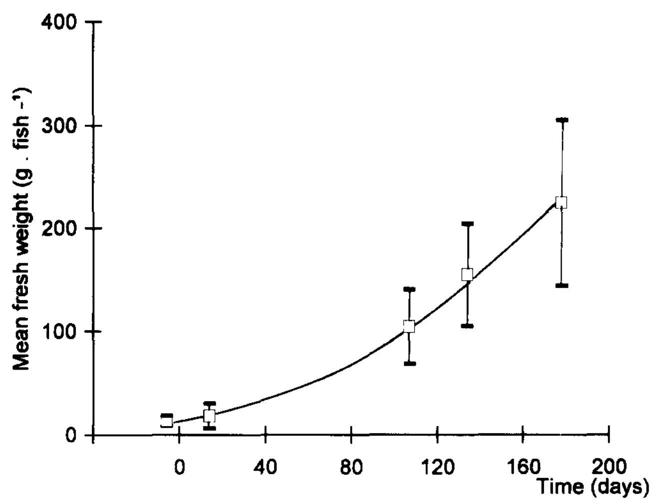


Fig. 2. Fish growth during the spring-summer period (bar = ±SD).

Table 2
Yield of the different genotypes of lettuce at the end of the winter trial

| Lettuce type | Cultivar | Fresh weight (g) of the head ± S.D. | % of control |
|---|---|---|---|
| Butterhead | Judy | 325 ± 27 | 100 |
| | Musette | 199 ± 22 | 73 |
| | Ardente | 167 ± 15 | 73 |
| Crisphead | GL 659 | 171 ± 70 | 112 |
| | Malika | 162 ± 47 | 79 |
| | Salinas | 101 ± 36 | 60 |
| Cos | Parris Island | 166 ± 58 | 82 |
| Loose-leaf | Red head | 146 ± 43 | 66 |

Crop without extra lighting (five plants of each cultivar).
Control, conventional recycled hydroponic culture.

Table 3
Input of water and mineral complement to the ecosystem during the three experimental periods

| | 1° period (autumn) | 2° period (spring–summer) | 3° period (winter) |
|---|---|---|---|
| Duration (days) | 104 | 153 | 68 |
| Water input ($m^3$) | 8.1 | 31.6 | 6.1 |
| *Minerals added (g)* | | | |
| N | 36 | 54 | 0 |
| P | 183 | 808 | 96 |
| K | 203 | 1067 | 121 |
| Mg | 3 | 28 | 8 |

112 % of that obtained in the conventional recycled hydroponic technique (Lesaint, 1987). These results demonstrate the different behaviour of cultivars growing in a dilute nutrient solution.

### 3.1.3. Water and mineral nutrient input

During the three experiments, the daily water renewal varied from 0.1 to 0.3 % if expressed as a percentage of the total water recycled, or from 3.5 to 9 % if expressed as a percentage of the total volume of the system (2.25 $m^3$). The daily water renewal depended on water consumption by the plants, and therefore on the climate (Table 3). It agreed with results obtained in recycled systems already described: 7 % in MacMurty et al. (1990), 6.6 % in Lewis et al. (1978) 6 % in Sutton and Lewis (1982) and 2.6 % in Watten and Busch (1984), expressed as a percentage of the total volume of the system.

Rakocy (1980) points out that water consumption in a recirculating system with plants is 600 times less than in a conventional raceway. Therefore, pollution of the external medium by fish effluent is greatly reduced, especially as its nitrogen content (mainly nitrate) is depleted by plant assimilation. As to plant requirements (tomatoes in the spring–summer period), the mineral supply was drastically lowered except for phosphorus (Table 3 and Fig. 3) when compared with a conventional recycled hydroponic culture (Lesaint, 1987). So vegetable production in recirculating system was inexpensive.

## 3.2. Nitrogen dynamics in the ecosystem

### 3.2.1. Changes in the nitrogenous compounds of the recirculating water

*3.2.1.1. During the spring–summer and winter periods.* Water from the decanter was sampled three times a week at noon (3 h after the first feeding).

During the spring–summer experiment with tomato plants (Fig. 4(a)) $NH_4$ content (50–170 $\mu mol\ l^{-1}$) and $NO_2$ content (0–50 $\mu mol\ l^{-1}$) remained largely below the toxic values for fish (Quilleré et al., 1993). Two weeks after plant setting (Day 21), the $NO_3$ content in water decreased to a level near 1 mmol $l^{-1}$ related to an active growth of the plants. Similarly nitrate content fell abruptly in relation to the setting up of 40 new plants (Day 128). On Day 140, all the water was renewed after the sudden death of 93 fish.

The winter period with lettuce plants (Fig. 4(b)) was characterized by a lower $NH_4$ (2–30 $\mu mol\ l^{-1}$) and $NO_2$ content (4–12 $\mu mol\ l^{-1}$) in recirculating water, but a higher $NO_3$ content which increased up to 5 mmol $l^{-1}$.

During these two periods, the performance of the bacterial filter was high as neither $NH_4$ nor $NO_2$ accumulated in the water. The evolution in the $NO_3$ flow expressed the system's equilibrium. So in spring–summer, the increased fish excretion (as the fish feeding increased) was

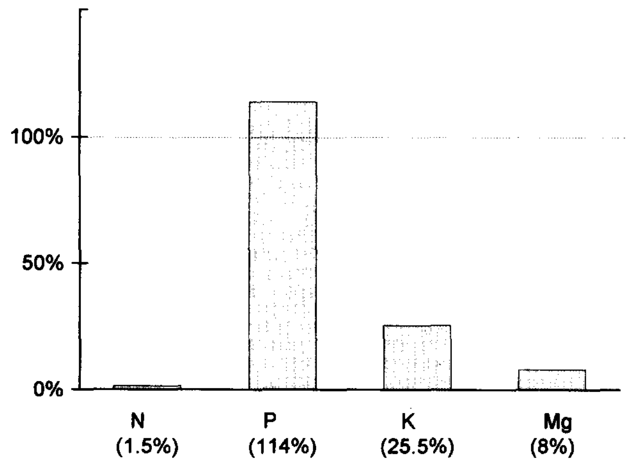


Fig. 3. Minerals supplied to the tomato plants during the spring-summer period as percent of the supply to a conventional recycled hydroponic culture.

balanced by the requirements of the tomato plants but in winter the slow growth of the lettuce plants did not balance the constant fish excretion though lower than in spring–summer.

*3.2.1.2. During a nycthemeron.* Recirculating water was sampled on 10 May (Day 99) during the spring–summer period on and after 5.30 a.m. (G.M.T.) over 24 h. The water samples came from the decanter (D) and the tank collecting the effluents from the hydroponic troughs (H).

In D, $NH_4$ content increased with food supply and decreased during the night period, the maximum for $NO_2$ occurred later than for ammonia (Fig.5).

In H, the lowered contents in $NH_4$ and $NO_2$ showed the efficiency of both the bacterial filter and the hydroponic troughs. They reduced the fluctuations of the nitrogen forms and lowered the toxic compounds for fish, so $NO_2$ content fell nearly to zero in H.

During the day, $NO_3$ content in D and H ran roughly in a parallel direction and at a constant level (Fig. 5) indicating that fish excretion and plant absorption were balanced. During the night $NO_3$ absorption by plants decreased (Pearson and Steer, 1977; Le Bot and Kirkby, 1992) so the curves became closer to each other and the $NO_3$ content increased in the whole system.

*3.2.2. Estimate of nitrogen flows in the ecosystem*

Fig. 6 represents a schematic view of nitrogen flows in the ecosystem. The nitrogen mass balance of the ecosystem (Table 4) was established for the three periods of functioning on the basis of the results obtained for the biological productions (Table 1), the water and mineral complement input (Table 3) and the nitrogen content of the animal and plant production (Table 5).

The nitrogen assimilated by fish varied from 18 to 32% of the total N feed. The 32% value was obtained with growing fingerlings, which is the normal functioning procedure for the fish compartment. On tilapias bred in conventional systems (with a similar protein diet and growth period) the assimilated nitrogen amounted to 40–43.2% of nitrogen ingested by fish (Siddiqui et al., 1988; El Sayed, 1990). When assimilated nitrogen referred to the total nitrogen in the feed supply, smaller values were attained: in recirculating systems without plants Suresh and Lin (1992) obtained less than 20% of assimilation on a male population, and in recirculating systems with plants Rakocy (1980) obtained 31 % with mixed populations and Zweig (1986) 37.4% on a male population.

Thus, 68% (for spring–summer) or more of the nitrogen feed was released in the breeding medium. Nitrogen feces represent about 10 % of the ingested nitrogen (Luquet, personal communi-

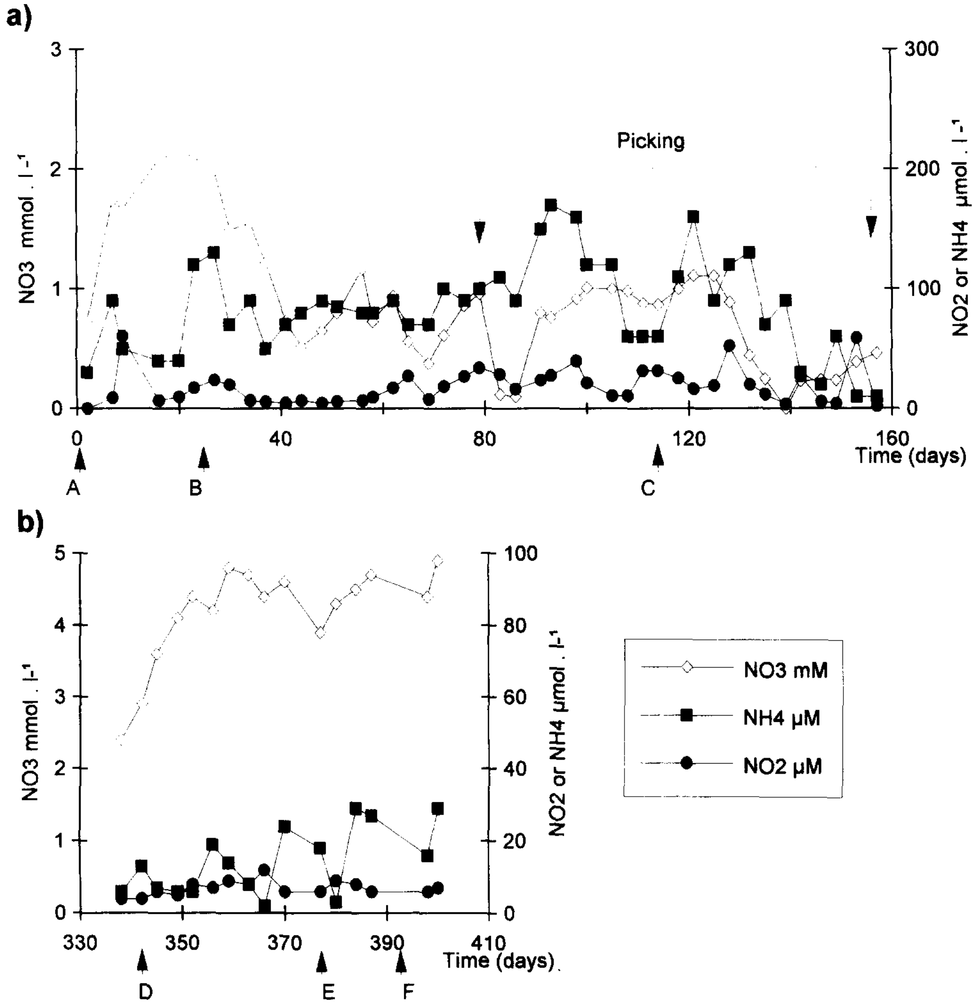


Fig. 4. Evolution of the nitrate ($NO_3$), nitrite ($NO_2$) and ammonia ($NH_4$) contents in the recirculating water (decanter). a. During the spring-summer period with tomato plants. A = Introduction of 80 plants (D6); B = Topping of half the plants (D28); C = Harvest of 40 plants and introduction of 40 new plants (D114). b. During the winter period with lettuce plants. D = Introduction of 176 plants (D343); E = Harvest of 96 plants (D377); F = Final harvest (D393).

cation, 1990). The main part of nitrogen excreted by the fish is recovered in the breeding water as $NH_4$ (30–40% of ingested nitrogen in carp according to Kaushik, 1980). In the present ecosystem with a nitrifying bacterial compartment, $NO_3$ is the predominant nitrogen form in circulating water.

Nitrogen assimilated by plants during the three trials (autumn, spring–summer and winter) amounted to 31%, 28% and 8.5% respectively of the total nitrogen input (Table 4). These levels were similar to nitrogen assimilation by fish (18–32%) because in plants all the nitrogen absorbed is trapped in their tissues, unlike fish. Results obtained by Rakocy (1980) were much lower and reached 7–8.6%; they increased up to 10–14% when referring to the non-assimilated part of fish feed. On the same basis, the nitrogen assimilated by the plants in the present system (minus nitrogen in the tap water and in the mineral complement) represented in the three trials 30.5%, 35% and 7.5% respectively. So in this

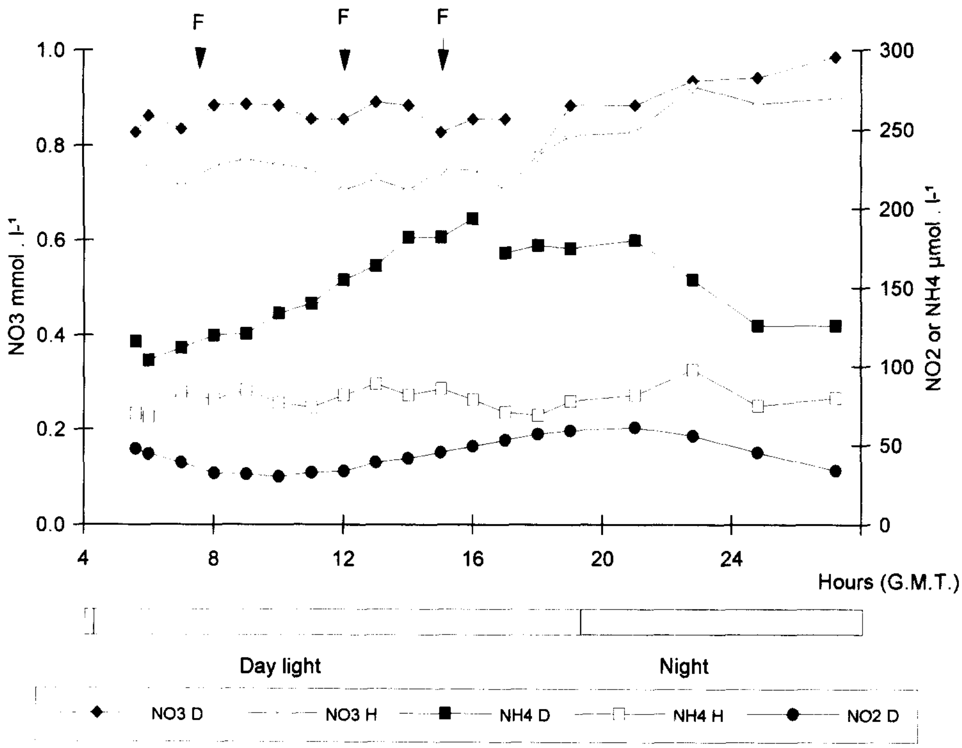


Fig. 5. Nitrogenous compounds in the recirculating water during a day-night cycle (Day 99 of the second period). Ammonia ($NH_4$) and nitrite ($NO_2$) content ($\mu$mol $l^{-1}$). Nitrate ($NO_3$) content (mmol $l^{-1}$). F=Feeding time; D=Decanter; H=Hydroponic trough.

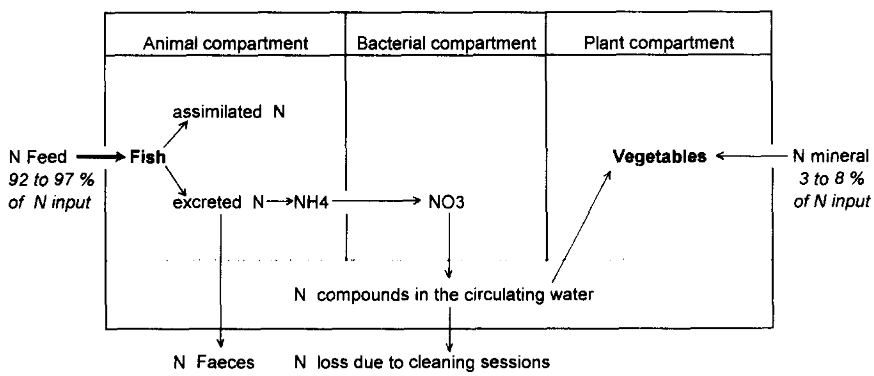


Fig. 6. Scheme of the nitrogen flow through the artificial ecosystem.

ecosystem, it appears that growing plants could recover 35% of the nitrogen waste. These results point out the important role of plants in waste water purifying and the excellent performance of the plant compartment when it is well proportioned to the fish compartment and supplied with a nutrient solution to optimize its mineral nutrition (Quilleré et al., 1993). The efficiency of the system could be improved if the water flushed away during each periodical cleaning (nitrogen loss Table 4) was reintroduced after preliminary treatment.

Table 4
Nitrogen mass balance in the artificial ecosystem at the end of each experimental period

| | 1° period (autumn) tomatoes | | 2° period (spring–summer) tomatoes | | 3° period (winter) lettuces | |
|---|---|---|---|---|---|---|
| *Nitrogen input* (g N) | | | | | | |
| Feed intake | 803 | | 3202 | | 895 | |
| Mineral complement | 36 | | 54 | | 0 | |
| Water[a] | 34 | | 133 | | 26 | |
| *Nitrogen output* (g N and % of N input) | | | | | | |
| Fish | 146 | 18%[b] | 1015 | 32%[b] | 185 | 21%[b] |
| Vegetables | 269 | 31% | 949 | 28% | 78 | 8.5% |
| *Nitrogen remainder* (g N and % of N input) | | | | | | |
| Water | 117 | 13.5% | 0 | 0% | 139 | 15% |
| *Nitrogen loss* (g N) | | | | | | |
| Faeces and water lost during cleaning sessions | 341 | | 1425 | | 516 | |

[a] Tap water contained nitrate (4.2 g $N.m^{-3}$).
[b] N output for fish expressed as % of N feed.

Table 5
Nitrogen content in fish and plant productions

| N % F.W. | | | N % D.W. | | |
|---|---|---|---|---|---|
| Whole fish | Tomatoes fruits | Lettuces heads | Tomatoes non-edible parts | | Lettuces non-edible parts |
| 2.5% | 0.13% | 0.21% | 3.2% (1° period) | 2.4% (2° period) | 5.1% |

The reduced nitrogen assimilation during lettuce cultivation (Table 4) is not due to poor plant growth but to an insufficient number of plants with regard to the fish population. Consequently nitrogen waste level in the water increased to 15% of the nitrogen supplied. This points out the necessity to define the adequate ratio between plant and fish population for each type of plant culture. But the most worrying source of imbalance in the ecosystem is the synchronisation of the biological cycles in order to have plant nitrogen absorption equal or in excess of nitrogen supply by fish. This is difficult to achieve as biological cycles are not of the same duration and the nitrogen requirements of plants are not in proportion with nitrogen excretion of fish. For example in the first trial (Quilleré et al., 1993) plant topping of the tomato culture resulted in a decrease of nitrogen absorption, whereas nitrogen supply by fish remained constant. This difficulty could be overcome in using systems associating several fish tanks with several vegetable crops. In a small system only the plant compartment can be regulated, in a two-step manner: (1) staggering the crop to maintain a high nitrogen demand; (2) adding nitrate to the mineral complement when nitrogen excreted by fish becomes too low.

## 4. Conclusion

The results obtained in this system of associated production emphasize the following positive points:

(1) fish production at the same level as in intensive breeding in non recirculating water systems and an important saving in water.

(2) reduced environmental pollution with a concomitant valorization of the fish breeding waste (in particular nitrogenous compounds) through an edible plant production.

(3) vegetable production of market quality, lower than in a conventional hydroponic crop but with a large saving in fertilizers, notably nitrogen.

(4) flexibility in the location of the system, the two constraints being the need for a water reserve and an energy source for the circulation of water.

However, the management of this system requires substantial multidisciplinary maintenance, as animal or plant pests or technical failures linked to energy or water supply can break the equilibrium between the different compartments. Indeed natural ecosystems possess compartments of such dimensions that sudden transfers of flux between the compartments are buffered. Artificial ecosystems present a limited potential for self stability (Bréchignac and Wolf, 1990) due to their restricted dimensions, the presence of few species and a low genetic and environmental variability (Richter and Diekkrüger, 1990). So frequent controls are needed to prevent any possible dysfunction.

Practical applications can aim at different objectives:

(1) In sahelian countries of Africa where water and energy resources are scarce, this combined system of intensive fish and vegetable production functioning with solar energy can contribute to the development of local food production. An experiment of this type is being carried out in Niger (Kane, personal communication, 1990).

(2) In countries where water supply is not limited pisciculture releases large volumes of polluted water into the environment. Hydroponics in single pass or in recirculating systems like this could be used (Lewis et al., 1978) if environment regulations become more restrictive.

(3) Finally, on a reduced scale, as a small unit of autonomous production with a high level of waste recycling, this system could be adapted to confined surroundings such as spaceships.

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