

INTRODUCTION & OBJECTIVES

Integrated MultiTrophic Aquaculture (IMTA) is based on an **ecosystem approach** framework, where the farming of aquaculture species from different trophic levels with complementary ecosystem functions allows one species' uneaten feed and wastes, nutrients and by-products (in particulate and dissolved forms) to be recaptured and converted into fertilizer, feed and energy for the other species, taking advantage of synergistic interaction between species. Moreover, the combination of IMTA with hydroponics allows to minimize water consumption, avoids the discharge of effluents enriched in dissolved nitrogen (N) and phosphorus (P) from IMTA into ground or surface waters, and decreases the need of using crop fertilisers of mineral origin made from depleting natural resources by recovering the nutrients from wastewater (converting fish excretion into high-value products for plants).

The present study, conducted in the frame of **HortiMED H2ozo PRIMA Project** (Grant Number 1915) funded by the European Union, was aimed at **evaluating the feasibility of combining Integrated MultiTrophic Aquaculture (IMTA)** including the production of Nile tilapia (*Oreochromis niloticus*), mullet (*Liza ramada*), crayfish (*Procambarus clarkii*), clams (*Aspatharia chaiziana* and *Aspatharia*, family *Iridinidae*) and silver carp (*Hypophthalmichthys molitrix*) **with hydroponic horticultural production** (red and green leaf lettuce, chili and bell peppers, cucumber, eggplant, mallow, watercress and celery) using (i) Nutrient Film Technique (NFT) and (ii) Floating Raft System (FRS) **to maximize nutrient cycling** resulting from culturing plants and aquatic animals.

MATERIALS & METHODS

The study was conducted at the Fish Research Station, El-Kanater El-Khayria, of NIOF (Qalyubia Governorate, Egypt). The experimental project area consists of three plastic greenhouses with a simple metal structure: (i) Greenhouse 1- A greenhouse of 140 m² (20m x 7m) with 2 NFT hydroponic units and 2 FRS hydroponic systems; (ii) Greenhouse 2- A greenhouse of 210 m² (30m x 7m) with a hydroponic FRS unit and a traditional unit (soil-based), and (iii) Greenhouse 3- A greenhouse of 240 m² (10m x 24m) with 4 concrete ponds of 40m³ for the production of different aquatic species.

SYSTEM SET UP & OPERATION-The system relies on five water pumps and manual ball valves to manage the water flow within the system. The fresh water is pumped from the well water source by a self-priming pump (Pump No.1) to two water tanks at a flow rate of 10 m³/h. Then it flows by gravity to the Greenhouse 3 to fill in the Nile tilapia pond (first pond), and moves to the remaining aquaculture ponds by means of a series of monks. The other inlet manual valves of Greenhouse 3 are only for emergency use. The sedimentation pond acts as a mechanical filter where a significant part of the solid wastes is captured. The water from the sedimentation pond is pumped to a large biological filter of 6m³ where ammonia is oxidized to nitrate. After the biological filter, the water goes through a sand filter, and then is pumped either to the Greenhouse 2 (Pump No.3) or the Greenhouse 1 (Pump No. 4). Pump No.3 & Pump No.4 can work simultaneously, but normally only Pump No.4 works continuously to provide a continuous water flow in the water recycling system (Aquatic Ponds → NFT & FRS units of Greenhouse 1 → Aquatic Ponds). Within the hydroponic units, the water moves by gravity through the FRS & NFT units at a mass flow of around 2.7m³/h for each FRS unit and around 1.3 m³/h for each NFT unit. The water ends in a sump pond of 3 m² (1.5m² used for sedimentation and 1.5m² acting as biofilter) whose goal is to reduce the total amount of organic matter through sedimentation and to reduce, if any, the remaining ammonia group. Inside the Greenhouse 2, the water flow is diverted to the FRS or to the Traditional Soil Culture (TSC) unit via manual valves, and finally goes to an earthen pond of 700 m² or through an outlet drain.

A closed aeration network distributes air through air nozzles and nano-hose discs to the different units of the system. The aeration network relies on 2 air blowers (Siemens & SCHMALZ) that operate one by one alternatively with an interval of 30 minutes by means of automatic timers 24h/day. The system is powered by combining grid power and solar energy. The solar panels with a total capacity of 7kW, provide enough energy to operate the water pumps and the air blowers from 8:00 am to 3:00 pm.

AQUATIC SPECIES- 450 Nile tilapia fingerlings with an initial weight of 30±6.37g were placed in the first aquatic pond on 1 May 2021. On day 30, the polyculture of crayfish (165 crayfish with an initial body weight of 16.19±3.11g) and mullet (500 fingerling with an initial body weight 1.14±0.74g) was started in the second aquatic pond. On day 60, 650 silver carps with an initial weight of 3.65±2.99g were introduced in the fourth aquatic pond (sedimentation pond) to avoid the proliferation of algae. On day 90, 176 freshwater clams with an initial body weight 210±61.77g were introduced in the third pond. Aquatic animals were stocked in ponds according to the availability of their seed.

The only aquatic species artificially feed was the tilapia. Tilapia were fed an experimental diet (30% Crude protein, 7% lipid and 18 MJ/kg gross energy) at approximately 3% of body weight 6 days per week, three times a day (08:00, 12:00 and 15:00 h). Feeding rate based on tilapia biomass was corrected every three weeks by taking a random sample of at least 25% tilapia and weighting it.

CROPS- Seven vegetables were selected for comparative purposes based on their commercial importance, their difference in relative growth rates and their tolerance to different concentrations of minerals: 1- lettuce (red & green leaf), 2-cucumber (summer species), 3-eggplant, 4-pepper (bell & chilli), 5-mallow, 6-watercress and 7-celery. The crop trial protocol included TSC and hydroponic systems (NFT & FRS) for comparative purposes. The crops were sequentially grown so that total plant production was balanced with fish production, as depicted in Table 2. An Integrated Pest Management approach was followed, using traps with pheromones and adhesive yellow tape traps as physical control methods, performing routine pest monitoring, and employing ladybird beetles as biological control agents.



Figure S1: FRS and NFT units of Greenhouse 1.

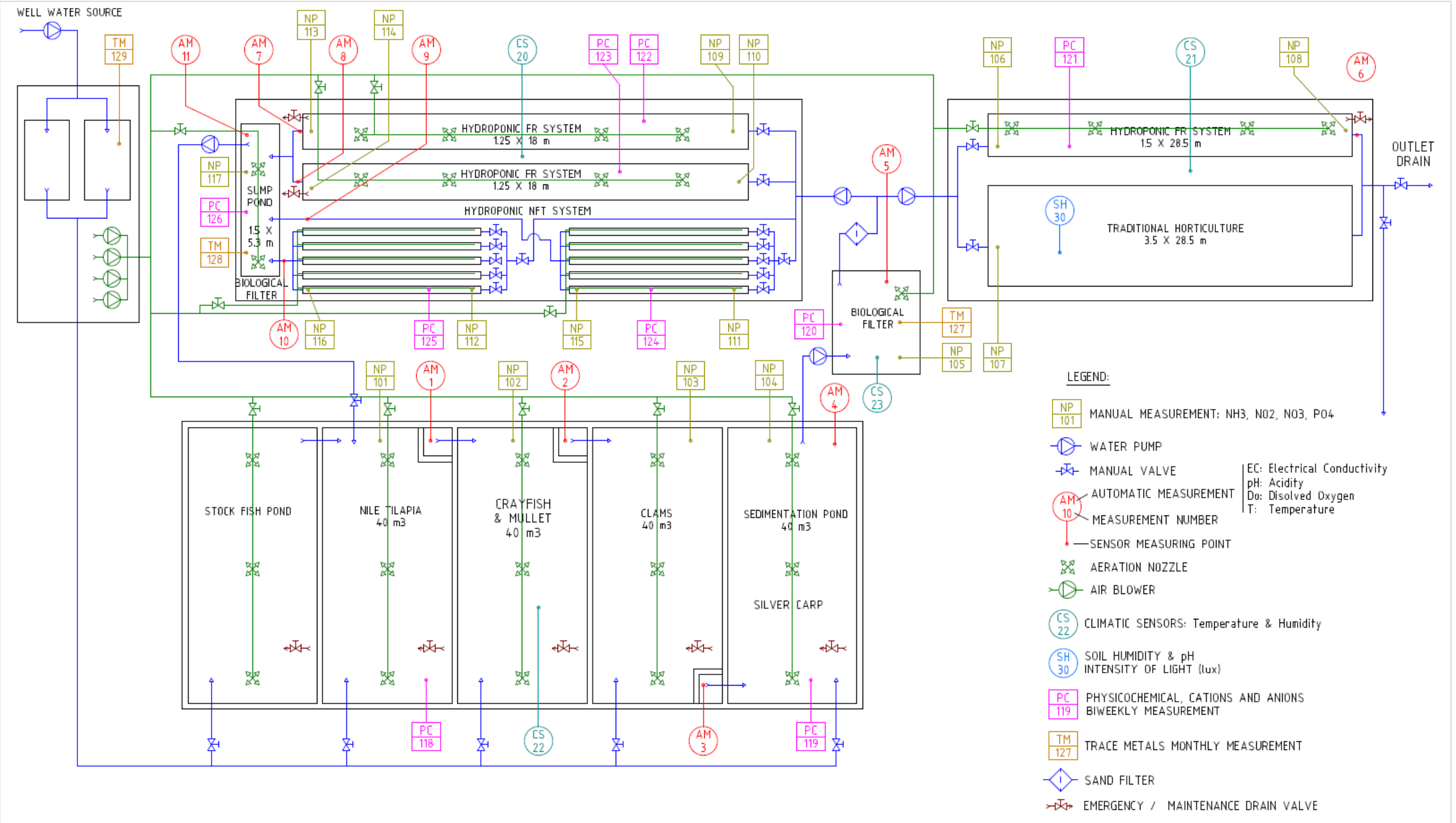


Figure S2: Blueprint of the experimental greenhouses (water flow-blue; airflow-green; drainage system-dark red).

To correctly manage the IMTA system **REGULAR MONITORING OF WATER AND ENVIRONMENTAL CONDITIONS** was conducted through a combination of:

- IoT system (WiFISH from ReNile)**, consisting of 11 sensing nodes (named AM in Figure 2) that measure pH, Dissolved Oxygen (DO), Electrical Conductivity (EC), water and air temperature and Relative Humidity (RH) and automatically calculate Total Dissolved Solids (TDS), salinity and percentage of oxygen saturation. All the parameters are recorded every 30 seconds and periodically sent to the HortiMED Data Capture System.
- Offline sensors** to monitor soil moisture, soil pH and light intensity in the traditional horticultural land area of Greenhouse 2 (named SH in Figure 2).
- Water sampling and laboratory analysis** of: 1-Weekly monitoring of Nitrogen (Ammonia, NH₃+; Nitrite, NO₂⁻; and Nitrate, NO₃⁻) and Phosphorous compounds (Phosphate, PO₄³⁻) at 17 sampling points; 2-Biweekly monitoring of physicochemical parameters (Carbonate, CO₃²⁻ and bi-carbonate, HCO₃⁻; TDS; Biological Oxygen Demand-BOD and Chemical Oxygen Demand-COD), major cations (Ca, Mg, K, Na) and major anions (F, SO₄) at 9 sampling points; and 3-Monthly monitoring of trace metals (Al, As, Ba, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, Se, Sb, Sn, Zn) at 3 sampling points (named NP, PC and TM, respectively, in Figure 2).
- HortiMED digital FieldBook**: a digital FieldBook APP has been developed to ease the recording of non-automatic data, including the measurements of offline sensors; laboratory analysis; and relevant data on crop & aquatic species growth (Figure 3).

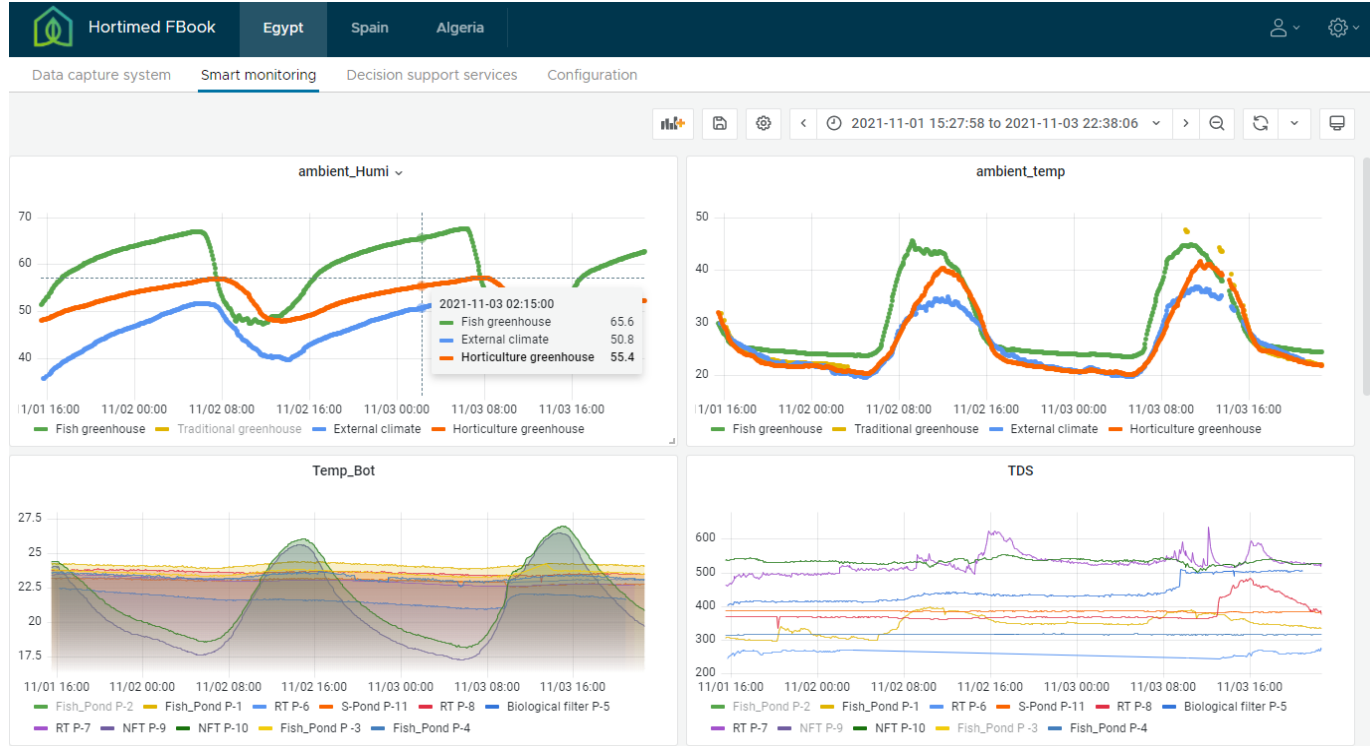


Figure S3: HortiMED digital FieldBook App displaying IoT system data.

NUTRIENT CYCLING- The main input of N and P in the system is the fish feed. Once the fish feed is added to the system, a substantial part of it is eaten by the tilapia and either used for growth and metabolism or excreted as soluble and solid faeces (m. Eck et al. 2019). Within the IMTA system (Figure 4) the uneaten feed, faeces and soluble excretions are recaptured by the subsequent extractive aquatic species (i.e. mullet, crayfish, clams and silver carp) which use them as nourishment, acting as living filters. Besides, the last pond acts as a mechanical filter where a significant part of the solid wastes is captured. The water from the sedimentation pond is pumped to a large biological filter of 6m³ where the excreted Total Ammonia Nitrogen (TAN) from fish is oxidized to nitrite and nitrate y ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, respectively, thus turning fish waste into accessible nutrients for plants. Nitrate and orthophosphate are assimilated by the plants' roots, improving the system's nutrient use efficiency, and thus ultimately creating better environment for aquatic species in the aquaculture tanks. In the present study, partial supplementary fertilization was conducted through foliar spraying to prevent Potassium (K), Magnesium (Mg) and Iron (Fe) deficiencies.

RESULTS

WATER USE EFFICIENCY (WUE)-The water consumed in the system is mainly due to two processes: (1) water consumed due to evaporation from the water ponds (around 19.22m³/week in winter and 35.22m³/week in summer) and (ii) water consumed due to plants' transpiration (3.22m³/week on average). The total water consumption of the system was 880.60 m³ for the whole cycle, leading to a WUE of 0.001467 m³ water/m² greenhouse/kg produced.

NUTRIENT USE EFFICIENCY (NUE)-The system is only partially fertilized to prevent micronutrient deficiencies. The total fertilizer consumption was 11.18kg, leading to a NUE of 1.86236E⁻⁰⁵ kg fertilizer/m²/kg.

BIOMASS PRODUCTION-The aquatic biomass of the system at the end of the cycle was 236.01 kg, and the total aquatic biomass gain was 179.83 kg (Table 1). The total crop biomass produced was 781.29 kg (Table 2), leading to a total system biomass (crop & aquatic species) of 1017.30kg.

FEED CONVERSIO RATIO (FCR)-Given that the tilapia is the only species fed (273.88kg feed/cycle), and the remaining aquatic species use as food source the wastes from the previous aquatic species, there is a cumulative improvement on apparent FCR values every time a new species is introduced in the system. The cumulative apparent FCR value of the system is 1.52 (Figure 5).

CONCLUSIONS

In HortiMED IMTA-aquaponics system **significant improvements** have been recorded in **NUE and WUE, net aquatic biomass production and FCR**, compared to traditional horticulture or aquatic monoculture systems. These results indicates that IMTA-aquaponics as a **bio-integrated food production system** is not only a successful method for the simultaneous crop and aquatic biomass production, but also a **suitable strategy for cycling nutrients and water**.

HortiMED will continue with the IMTA-aquaponics research in the experimental site during at least two production cycles, including: (i) the N and P balance for fish-culture ponds and different experimental hydroponic systems; (ii) optimizing plant crop yields for greenhouse vegetables and evaluating the production of other crop species (e.g. tomato and broccoli); and (iii) determining critical water quality parameters in the system to achieve optimal conditions for both fish and plants.

Table S1: Aquatic biomass production of the system.

	Nile tilapia	Grey Mullet	Crayfish	Clams	Silver carp	Total
Initial biomass (kg)	13.5	0.57	2.67	37.07	2.37	56.18
Gain (kg/cycle)	112.73	17.66	2.13	28.1	19.22	179.84
Final biomass (kg)	126.23	18.23	4.8	65.16	21.59	236.01

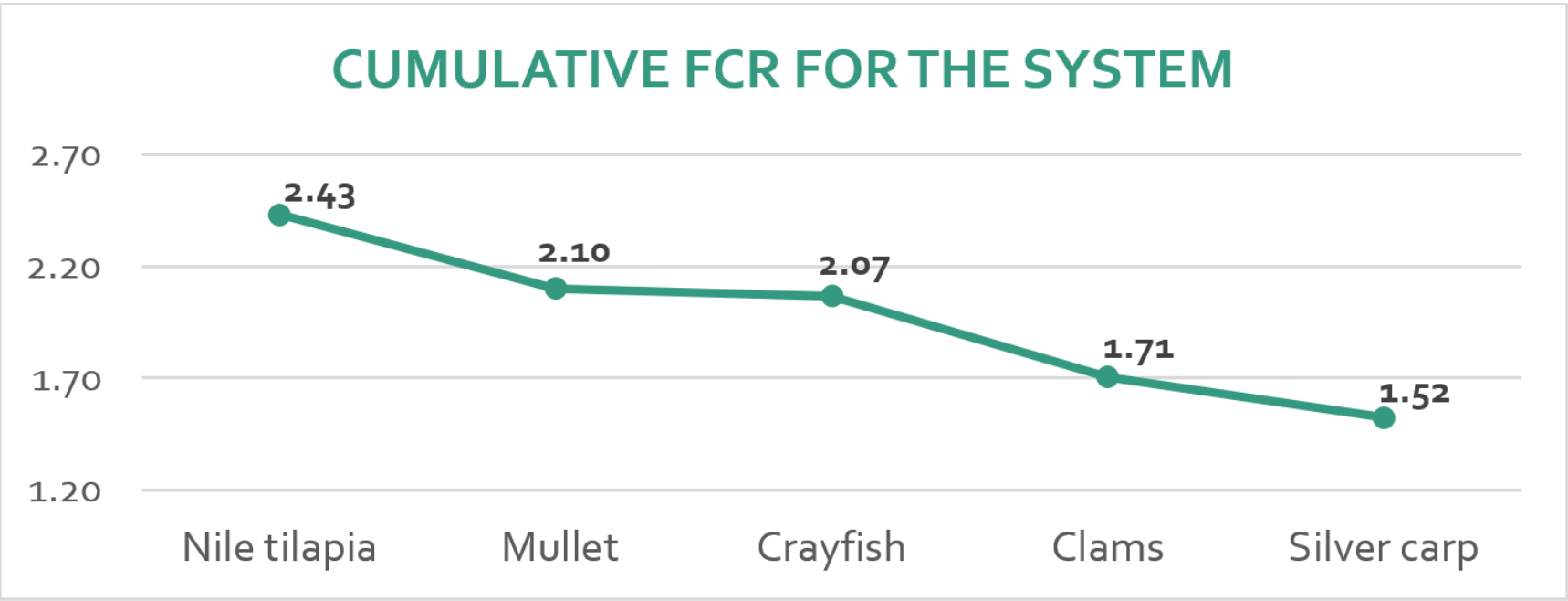


Figure S5: Cumulative FCR obtained by adding different aquatic animal species to the IMTA system.

Table S2: Yield and total production obtained for each crop under different cultivation system.

IMTA-Floating Raft System					
Crop	Transplanting date	Harvesting date	Area (m ²)	Total production (kg)	Yield (kg/m ²)
Chilli pepper	23/05/2021	24/09/2021	18	32.67	1.82
Cucumber	23/05/2021	31/07/2021	15	255.70	17.05
Bell pepper	30/05/2021	11/09/2021	9	9.39	1.04
Eggplant	15/06/2021	23/10/2021	6	3.50	0.58
Celery	18/07/2021	30/11/2021	3	4.50	1.50
Green leaf lettuce	18/07/2021	03/09/2021	18	28.79	1.60
Red leaf lettuce	18/07/2021	03/09/2021	18	24.64	1.37
IMTA-Traditional Soil Culture					
Crop	Transplanting date	Harvesting date	Area (m ²)	Total production (kg)	Yield (kg/m ²)
Chilli pepper	23/05/2021	24/09/2021	80	227.61	2.85
Bell pepper	30/05/2021	01/10/2021	20	55.80	2.79
Eggplant	15/06/2021	23/10/2021	6	29.50	4.92
Mallow	23/07/2021	20/11/2021	8	45.00	5.63
Watercress	13/07/2021	30/11/2021	8	18.50	2.31
Celery	18/07/2021	30/11/2021	3	6.00	2.00
IMTA-Nutrient Film Technique					
Crop	Transplanting date	Harvesting date	Area (m ²)	Total production (kg)	Yield (kg/m ²)
Green leaf lettuce	18/07/2021	03/09/2021	9	21.15	2.35
Red leaf lettuce	18/07/2021	03/09/2021	9	18.54	2.06