Deliverable 1.4: SIMTAP prototypes in selected countries (PU, M2)

(31 January 2021)

SIMTAP prototype in Italy

The SIMTAP system at UNIPI was designed according to climatic conditions expected inside a greenhouse, as reported in Deliverable 1.1-SIMTAP design. The system was built between July and September 2020 and is currently in operation for assessing and optimizing the mutual interactions of each unit. The layout is shown in **Fig. 1** and a few pictures of the system are shown in **Fig. 2**.

As traditional aquaponics combines fish and plant production in one simple water loop, some compromise is necessary to obtain optimal growth for the system as a whole unit. Decoupled aquaponics involves two recirculating loops for the physical separation of the fish and plant subunits. As the SIMTAP system is an experimental prototype, it was chosen to follow a flexible scheme capable of decoupling the main sections. In the system, an artificial sea salt water (Instant Ocean™, Aquarium Systems, France) was used to simulate the use of natural seawater. Raw water has a salinity of 0.5-1.0 g/L. Water evaporation is compensated with a mixture of fresh and saline water to maintain a salinity level around 25 g/L.

The SIMTAP project is based on the idea of recreating a closed environment in which multiple animal organisms (teleosts, polychaetes, bivalves, nudibranchs, etc.) and plants (higher plants) can live together. Separately, the production of microalgae destined to represent the primary source of carbon chains (carbohydrates, proteins, lipids, minerals, and vitamins) for animal organisms is foreseen. In practice, thanks to the administration of simple elements (macro- and microelements), light (natural and possibly artificial) and air (to provide both CO_2 and O_2), microalgae are produced. Through the flow of water and in an adequate measure, these are administered to the detritivore and filter feeding organisms (DFFO). The biomass thus produced, collected according to a program dependent on the respective growth performance, is then used to feed the fish housed in the fish production section. To this end, and given the experimental nature of the SIMTAP plant, the use of raw materials of external origin is also currently envisaged, such as commercial and/or bivalve feed (kindly supplied by the Blue Resolution Association; https://www.blueresolution.it/), polychaetes and other materials with characteristics similar to the DFFO produced in the plant.

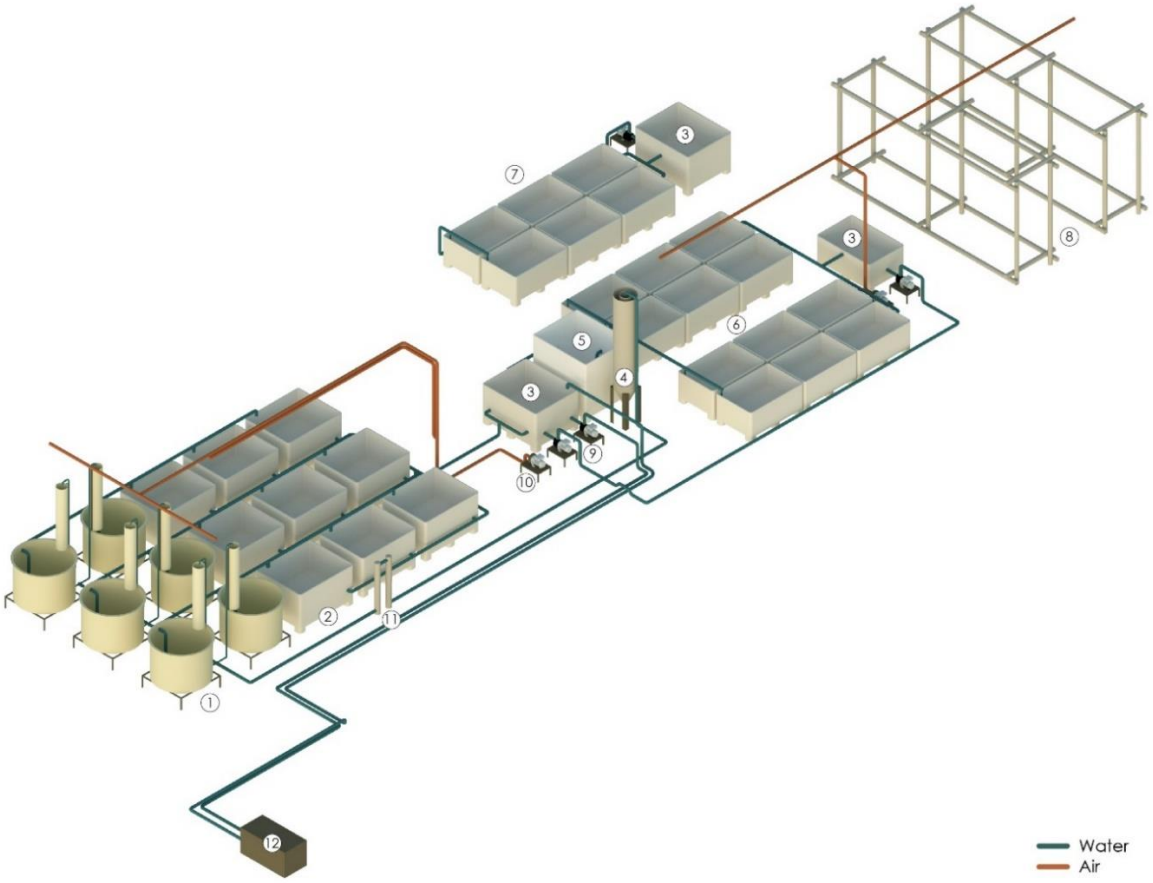

Fig. 1. 3D scheme of SIMTAP at UNIPI. Different components of the system are listed: (1) fish rearing tank, (2) FFDO tanks, (3) sump, (4) settler, (5) biofilter, (6) hydroponic and macroalgae section, (7) brackish water section, (8) microalgae section, (9) pumps, (10) blower, (11) UV steriliser, (12) reversible heat pump.

The wastewater from the fish breeding tanks (sea bream and sea bass are the species included in the project) is then conveyed to the DFFO section, where the suspended particulate is filtered by the bivalves or settles for the benefit of DFFO. The dissolved substances in solution (mainly nitrogen compounds and in particular ammonia/ammonium), after passing through the DFFO section, reach the filtering section and in particular the biofilter, where, thanks to nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*), the ammonia nitrogen is transformed into nitrite and then nitrate. Nitrates are absorbed and assimilated (i.e., used for protein biosynthesis) by macroalgae and halophytes (section 6), which then purify the water making it reusable for the breeding of animal

organisms, minimizing the need to drain water from the system to limit the accumulation of toxic substances and therefore reducing the overall water consumption of the system. The seawater recirculating in the system is prepared by adding a synthetic sea salt widely used in the aquarium sector, Instant Ocean (IO, Askoll Uno, Sandrigo, Vicenza), to the drinking water, at a concentration of 25 g/L (electrical conductivity, EC, of about 30 mS /cm).

Fig. 2. Some views of the SIMTAP system installed at the University of Pisa. From left to right, top to down, sections for seaweeds and halophytes (*Salicornia europaea* L.), fish, DFFO (mussel *Mytilus galloprovincialis*) and photobioreactors with microalgae (SEC_LI_ChL_1 strain and *Chlorella sorochiniana*).

Fish production section consists of six plastic (PP) tanks (circular section; capacity: 500 L; depth 0.7: m). The tanks are equipped with an automatic fish feeder. The section with detritivores and filter-feeder organisms (DFFO) consists of nine plastic tanks (PE, rectangular section; capacity: 500 L; depth: m 0.5) with a 8-10 cm layer of sand (Ø 0.4 - 1.2 mm) at the bottom. Macroalgae (seaweeds) and halophytic plant species are grown in two hydroponic (deep water culture, DWC)

subsections, respectively, with 12 or 6 plastic tanks (PE, rectangular section; capacity: 300 L; depth: m 0.4). Two sets of photobioreactors (100-L plastic bags) were also mounted for microalgae culture with brackish water or runoff water from greenhouse crops.

The system for water filtration/recirculation consists of: three recycle plastic tanks ("sump") for the collection and mixing of water with a capacity of 500 L (sump 1 and sump 3) or 300 L (sump 2); 1 vertical siphon settler, made of stainless steel; one plastic (PE) tank for biological filtration ("biofilter") with a capacity of 1,000 L. Temperature (T) is controlled with an external reversible heat pump (RHP; 2.5 kW), able to maintain a constant value of T during both cold and warm season. Pumping system consist of four water distribution circuits, three of them fed by one pump, as follows:

- 'RHP' circuit: sump 1> RHP> sump 1;
- 'Main' circuit: sump 1 > settler/skimmer > biofilter > hydroponic section > sump 2 > fish section > DFFO section > sump 1;
- 'Hydroponic II' circuit: sump 3 > hydroponic II section > sump 3.

The last circuit could be connected with the main circuit, if necessary.

The total water volume circulating into the system is around 13 m^3, depending on the pre-set water flow into the fish tanks, because the hydraulic head necessary for water circulation depends on the flow itself. Hence, being the water surface more than 25 m^2, each centimetre in water height means more than 250 L of stored water in the system.

Water biofiltration, disinfection (by UV lamps), degassing and aeration tasks are performed by separate units. Water aeration in all the tanks and photobioreactors is provided by two blowers, one for the fish and DFFO sectors and the other for the tanks with macroalgae (seaweeds) and halophytes, and the photobioreactors. The blowers can be operated separately. While in recirculating aquaculture systems (RAS) the water must be filtered to remove solids, total ammonia nitrogen (TAN) and CO_2, in the SIMTAP system at UNIPI the filtration is provided by DFFO and, therefore, no mechanical filtration has been applied. For the removal of fine solids (<30µm), a protein skimmer or foam fractionator might be used. These filters rely on agitation of water to create floating foam to which fine suspended solids bind; the foam is then removed from the water with a trap, which is under construction.

To maintain optimal T and safe pH and levels of dissolved oxygen, TAN, nitrite, nitrate (**Tab. 2**), the control system consists of many sensors continuously measuring water flux, T, EC, pH, and dissolved O_2, wired to a Programmable Logic Controller (PLC) capable of: 1) switching on/off all the pumps and blowers under a set of rules related to the measured parameter values; 2) sending alarms to the system personnel to cope with power crush or low oxygen level; 3) sending all the measured data of sensors and actuators, setting the frequency. In fact, the PLC is connected to the

web via a router with a SIM smart card. Besides, the partner UNIBO has built an integrated smart monitoring and control system (ISMaCS; Task 1.4) to: monitor and collect the data, to create local and remote database; to increase the operation precision, providing data to assess the environmental impact reduction. The ISMaCS is currently running, providing a double check of many parameters. The remaining critical parameters such as total ammonia nitrogen (TAN), nitrite and nitrate, are kept under control by chemical analysis.

Tab. 2. Critical parameters in the SIMTAP system installed at the University of Pisa

Parameter	Unit	Minimum	Maximum	Optimal*
Temperature	°C	15	27	23
Salinity	g/L	15	37	30
Dissolved Oxigen	mg/L	5	-	7-8
pH		6	8	7.7
TAN (NH_3 + NH_4^+)	mg/L	-	1	<1
Nitrite	mg/L			<50
Nitrate	mg/L			<300
Recirculation flow rate **	L/h	200	500	300

* For euryhaline fish (sea bass, sea bream and mullet) ** In the sections dedicated to fish production and DFFO.

SIMTAP in France

Rationale of the Case study

The rationale of the case study implemented by LML and INRA has been adapted after the first summer of experiment (2019). The general concept developed in France is presented on **Fig. 5** and is based on 4 principles:

1. Different species of different trophic levels can be associated in order to reuse the nutrient provided to fish by the formulated feed delivery. This strategy implies to develop specific technicity in the different species.
2. It is possible to rear fish (mullet and seabream) with vegetable formulated feed completed with co-products from IMTA loop (mussels mainly). Unfortunately, due to the low survival of mussel in coastal ponds, it has been decided to complete the fish diet by small wild shrimp from the pond system, and discarded mussel from local producers (in a perspective of circular economy).

3. It is necessary to adapt the water flow between the different compartments, in order to optimize the water residence time, the nutrient use and the O_2 and CO_2 flows. In this objective a specific model was built based on mass balance and material flow analysis.
4. Aggregation of knowledge. All the knowledge was associated to propose the design of the SIMTAP system. This design will be applied on the running system, which will be monitored in the task 2.2.

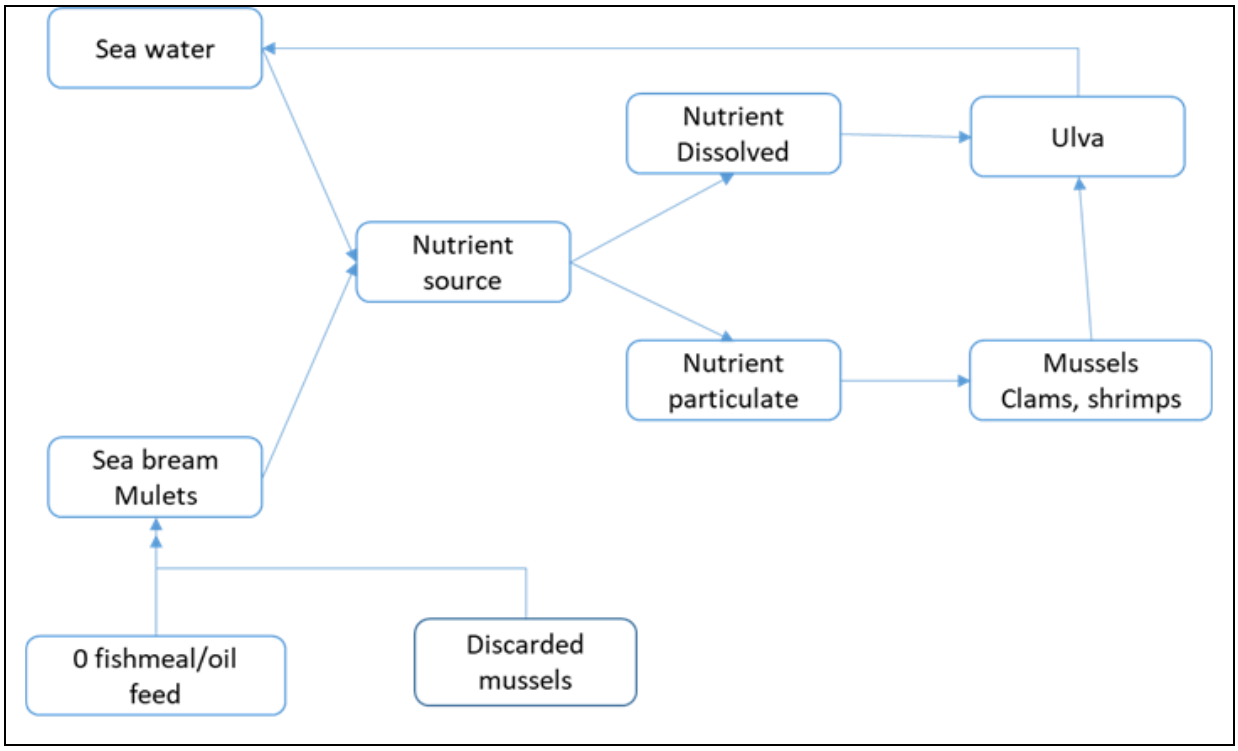

Fig. 5. Representation of principles of LML-INRA case study.

Description of the structure and running characteristics

The system is composed of five earthen ponds connected according to a cascade principle (**Fig. 6**). The first pond can be filled at high tide (over a tidal coefficient of 85) in pumping the water by airlift from the inlet water channel, separated from the ocean by a gate. This gate controls the depth of the water in the channel. The water depth of the ponds is determined by the level of the outlet pipe, connecting the last rearing pond (clams' pond) to the inlet water channel, for which an extension pipe is set up to avoid water inlet at high tide by this way. At low tide, the extension pipe is removed from the outlet pipe described just before and set on the outlet pipe connecting

the clams' pond to the ulvas' pond. In addition, the gate is closed, so that the airlift keeps pumping the water from the channel (now isolated from the ocean) into the first pond, which circulates in a loop, through the ponds until the channel. Thus, the only way for the outlet water is through the ulva's pond, when the water of the system is replaced during a high tide. To complete, an air blower provides air in each pond (except in ulva's pond) to insure a minimum concentration in oxygen, especially during summer. The indicators used to manage the replacement of the water into the system is the salinity, the depth of the water into the ponds (*e.g.* to compensate evaporation) and, if necessary, the concentration in oxygen.

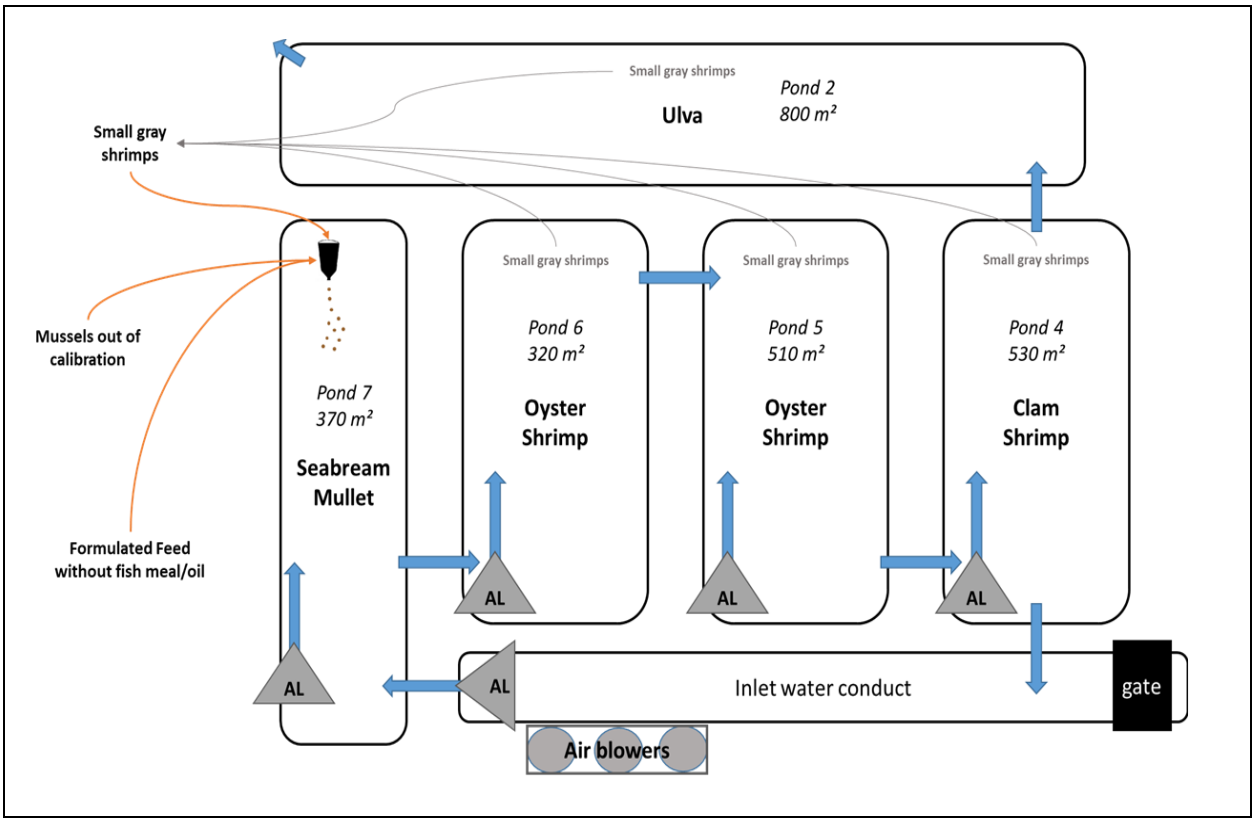

Fig. 6. Diagram of the experimental design of the SIMTAP system at INRAE-LML

Species stocking

In the first pond, gilthead seabream (*Sparus aurata*) was stocked at a density of 450 g/m² (3 fish/m², *i.e.* a total of 1400 fish). In the two following ponds, oyster (*Crassostrea gigas*) and shrimp were stocked at a density of 2 oysters/m² (mean weight of 45-50 g) and 2.5 shrimps/m² (at the

post-larvae developmental stage, mean weight of 0.5 g). In the following pond, clam (*Ruditapes decussatus*) and shrimp (*Penaeus japonicus*) were stocked at a density of 30 clams/m² (mean weight of 5 g) and 2.5 shrimps/m² (at the post-larvae developmental stage, mean weight of 0.5 g). The last pond was dedicated to macro algae (e.g. *Ulva sp*), expected to grow up in this compartment.

Gilthead seabream is the fed species, with a carnivorous diet, able to eat shellfish (e.g. mussels) and small gray shrimps and crabs. Oyster and clam are filter feeders of economic interest, able to eat suspended organic matter released by fish. Phytoplankton and small gray shrimp (*Crangon crangon*) settled and developed by themselves in each pond. The first one uses dissolved nutrients released by fish to grow up and is also a source of feed for filter feeders (oyster and clam). Small gray shrimp were trapped and introduced into the fishpond as live feed, to balance formulated feed in fatty acid and in protein required for fish development. The quantity available in small gray shrimp was not sufficient nor regular, that is why mussel out of calibration discarded from local producers, without economical value, was used (1/5 of the provided gross energy). Shrimp (*Penaeus japonicus*) stocked, in addition to its market value, has been chosen for its burrowing activity in the sediment, thus releasing nutrients into the water column and, therefore, available for phytoplankton growth. Furthermore, to enable phytoplankton growth and so to avoid competition with macro algae for nutrients and light, the second one was harvested in the ponds in which filter feeders are stocked.

SIMTAP in Turkey

A fully recirculated indoor system includes two main compartments: aquaponics including fish and halophyte/microalgae units and deposit feeder unit (**Fig. 7**).

Recirculating System: Although sea water (salinity 38-40 ppt), brackish water (salinity between 8 and 14 ppt) and fresh water (salinity 0.2-0.4 ppt) are available in the system, sea water of 38 ppt subjected to the eastern Mediterranean conditions is used in the SIMTAP implementation at MEDFRI.

The water is first filtered with a particle filter of 10 µm and stored in a sump tank following by ozonisation in a tank of 0.5 m^3 using a generator with a capability of 2 g per sec. In the system, water is pumped using a speed and flow controlled heavy-duty pump (0.75 kW) into fish growing tanks which is placed on a platform of 2.5 m height. Before reaching the rearing tanks, water is passed through a UV disinfection system (58 W; 846 mm lamp length). The outflow of fish rearing tanks is gravitationally drained to radial flow settlers. The outlet water is discharged from the upper level of the settler into a fluidized media bed filter containing 0.75 m^3 biomedia (500 m^2 per m^3) while settled solids to the deposit feeder units. The sand filtered outlet of DFFO units is also

transferred to the fluidized media bed filter. Water from the biofilter unit is gravitationally fed to the hydroponic unit to grow halophyte plants and microalgae as well as to reduce nutrient concentrations in the system. The outlet of the hydroponic unit is discharged to a protein skimmer to remove fine particulate organic compounds (1.1 kW; total length 1.80 m; reaction body 1.30 m; single venturi).

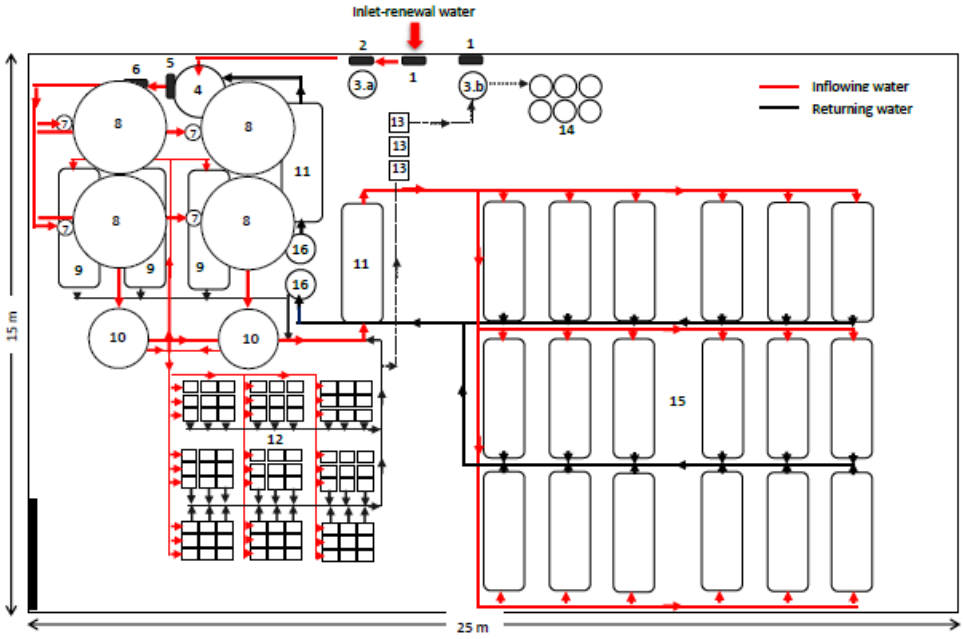

Fig. 7. Process flow diagram for SIMTAP system at MEDFRI (1. Particle filter with a capability of 10 µm, 2. Ozone generator (2 g per sec), 3. Ozonation tank (a-0.5 m^3; b-0.1 m^3), 4. Sump tank (3 m^3), 5. Water pump (a speed and flow controlled heavy duty pump; 0.75 kW), 6. UV lamp (58 W; 846 mm lamp length), 7. Degassing column, 8. Fish tank (5 m^3), 9. Sea cucumber tank (3 m^3 * 3 tanks), 10. Radial flow settler (1,5 m^3), 11. Fluidized media bed filter (tank 0.75 m^3; media 1000 m^2 per m^3), 12. Polychaete experimental unit (0,12 m^2 * 81 tanks), 13. Oxidation tank (0,1 m^3 * 3 tanks), 14. Microalgae unit (0.1 m^3* 6 plastic bags), 15. Halophyte unit (Deep Water Culture technique; 3 m^2 * 18 tanks), 16. Protein skimmer (1.1 kW; reaction body 1.3 m)).

A second fluidized media bed filter containing 0.75 m^3 biomedia (500 m^2 per m^3) is used following the protein skimmer. Finally, filtered and nitrified water is discharged back to the sump tank. A blower (50-60 Hz; 2.2-2.55 kW; maximum airflow 318-376 m^3 per hour) supplies air to fish tanks, halophyte, and microalgae units.

Fish unit: Fish culture is carried out in four 5 m^3 polyester circular tanks with 250 cm diameter and 100 cm depth. The tanks have dual drains from the bottom and edge. European sea bass and

gilthead sea bream juveniles will be stocked with a density of 7600 juveniles per m^3 and 8000 juveniles per m^3, respectively, during the adaptation stage (up to 5 g) of about 60 to 80 days at the next period. The applicability and performance of the system are testing for the on-growing stage from 5 g to market size with a density of 5-15 kg per m^3 for about 6 months. Daily water change is 7 times per day (1.15 m3 per hour for each tank). Water losses resulting from evaporation are renewed with a mixture of marine and brackish water to avoid a salinity increase.

Daily feeding rate (commercial feeds) is gradually decreased from 7% to about 1% of live weight depending on growth stages. Fish tanks were not illuminated and were continuously aerated with a blower. Fish material was supplied from both MEDFRI's hatchery and other local hatcheries.

Halophyte unit: Halophyte growing unit based on DWC technique with and without media was composed of 18 rectangular polyester tanks with a surface area of 3 m^2 (300*100*50 cm; length*width*depth) to ensure replicate data collection.

A water depth of 30 cm was provided in the tanks which means 900 L total storage capacity. Water flow rates at 2700, 1350, and 675 L per hour will be tested to investigate different hydraulic retention times (1, 2, and 4 hours, respectively).

The outflow of moving bed biofilter which takes the discharge of fish rearing tanks followed by radial flow settler gravitationally feds the halophyte growth unit.

There is not additional illumination except for daylight coming from a semi-transparent roof. *Salicornia* sp. which will be multiplied with seed germination will be grown in the unit. Species will also be identified through molecular techniques. The rafts and beds will be planted with a density of 100 seedling per m^2. The seeds and plants were collected from the coastal area of a lagoon adjacent to the Beymelek Unit of MEDFRI.

Microalgae unit: Microalgae will be cultured in transparent polyethylene bags with a capacity of 100 L. The effluents of the fish tanks and the protein skimmer will be tried as a media for microalgae culture. For this purpose, effluents will be filtered and then sterilized using a UV lamp and ozonization. Stored raw media will be intermittently added to the bags with and without micronutrient supplement. Both natural illumination with indirect sunlight from the transparent roof during daytime and artificial illumination using cold-white fluorescent tubes during night times will be tested, supporting also light/dark cycle. Mixing and carbon dioxide will be provided with aeration (if necessary pure carbon dioxide will be used).

After mass culture of 4-6 weeks, microalgae will be concentrated with centrifugation using a cream separator. Concentrated microalgae paste will be freshly used in the deposit feeder unit.

The growth performance of various microalgae species (*Tetraselmis suecica*, *Isochrysis galban*a, *Nannochloropsis oculata*, *Chlorella vulgaris* etc.) will be tested. The strains will be supplied from the microalgae culture collection of MEDFRI.

DFFO unit: The deposit feeder unit composes of two units including either polychaetes or sea cucumber. The polychaete experimental unit was installed using 81 rectangular high-density polyethylene tanks with a surface area of 0.1 m^2 (40*30*20 cm; length*width*depth) to ensure replicate factor tests. Some tanks were filled with a layer of 10 cm of sand (200-500 um particle size) which serves also as a slow sand filter while some were not filled with any substrate. The water level was supplied at 5 cm above the sand surface. To determine the tidal impact on feeding and growth, the outflow was maintained in both 15 and 5 cm of the tanks. The Polychaete unit was continuously fed by fish wastes discharged from the solid outlet of the radial flow settler. In this unit, different factors such as stocking density, feed source and hydraulic loading rates will be tested. Juvenile polychaetes with about 2 and 3 g will be stocked at densities of 200, 500 and 1000 individual per m^2. Hydraulic loading rates of 1.0, 2.0 and 4.0 L per m^2 per minute (146, 292 and 584 L per hours, respectively) will be tested. Various feed sources will be used: i) only fish waste, ii) fish waste plus a commercial fish feed, and iii) fish waste plus commercial fish feed plus microalgae paste.

Nereis sp., will be collected from natural populations of the coastal area of the Aegean Sea, will be cultured in the polychaete unit. Species will also be identified through molecular methods.

The sea cucumber unit was composed of two rectangular polyester tanks with a surface area of 3 m^2 (300*100*50 cm; length*width*depth). The tanks will be filled with a layer of 10 cm of sand (100-500 um particle size). Water level will be maintained at 30 cm above sand surface and feed will be supplied from the wastes of the bottom outlet of radial flow settler.

Preferably, *Holothuria tubulosa* will be collected from natural populations of the coastal area of the Aegean Sea and grown in the unit.

SIMTAP in Malta

The MAFA SIMTAP system will be located in a greenhouse at the Agriculture and Innovation Research Hub in Għammieri Marsa. The system will be hosted on a flat area of 8m x 24m a total of 192 m^2. The only component that will be at a different level is the micro-algae unit that will host a 10 m^2 area on a separate level. The system will be filled with ground brackish water. The desired salinity will be reached by adding a specific amount of salts to get an average salinity of 35 g/L.

Ground water of salinity between 3.0 and 5.0 mS/cm will be first filtered with a particle filter of 10 µm and stored in a sump tank. In the system, water will be pumped using a speed and flow-controlled pump into the 3 fish tanks (500 L). Before reaching the rearing tanks, water will be passed through a UV disinfection system and a heath pump so that ideal water temperatures are kept.

This SIMTAP system is decoupled aquaponics that involves two recirculating loops for the physical separation of the fish and plant subunits, this will lead us for a better monitoring and individual plant, fish growth. The main component of the SIMTAP are the aquaculture units, then water will flow through the DFFO units, afterwards to the hydroponic units for macro-algae.

The Aquaculture units consists of three 500-L tanks for fish growth. These tanks will be made of polyester (or PP) circular tanks (100 cm diameter and 70-80 cm depth). The tanks will have dual drains from the bottom and edge. Gilthead sea bream juveniles will be stocked with a density ranging from 1 to 10 kg/m^3 during adaptation stage. Similar growth performances trials will be carried out using the same fish species but with higher body weight, with density ranging from 10 to 20 kg/m^3.

Within the deposit/filter feeder unit, a multi-trophic chain will be tested with “Polychaetes” and clams. Fish wastes and micro-algae will feed these organisms. A main target in the design of the tanks, as well as the inlet- and outlet structures is to make sure that the tanks have settling properties for small particle size. The water hydraulics are such that feces are moved towards the bottom (sand) in a matter of minutes. The fish tank spills out to 3 polychaetes & clams tanks, cubic or cylindric -shaped, flat bottom with a Volume of 400-500 L, a side length of 1.0m, and a Depth of 0.6m (Water height = 0.4m + Sand height = 0.1m + free height = 0.1m). The tanks will be filled by a layer of 10cm of sand (50-250 um particle size) which also serves a slow sand filter. Water level will be supplied at 40 cm above sand surface. Within the SIMTAP fully integrated section, macro algae (i.e. *Ulva lactuca*) will be grown in small tanks with ~40 cm depth of marine water. This growing unit will be composed of rectangular tanks with a surface area of 10 m^2 (50 cm water depth) to ensure a large enough volume for *U. lactuca* growth for nitrogen removal. The hydroponic units will be partially decoupled for growing halophytes plants. Halophyte growing unit based on the DWC technique without media will be composed of rectangular polyester (or PP) tanks with a surface area of 10.0 m^2 to ensure triplicated data collection. Different plant species will be tested to investigate growth rate at different salinity content, due to the decoupling of the halophyte loop from the main SIMTAP system. Microalgae will be cultured in transparent PE or PVC Lay Flat Hose Pipe with a capacity of 100-200 L. Stored nutrient solution will be intermittently added to the bags. Natural illumination with indirect sunlight from the transparent roof during daytime. Mixing and carbon dioxide will be provided with aeration. Total area dedicated to this unit is 10 m^2 and it will be placed on a higher level.