Project Number: 18110-2

Project Acronym: SIMTAP

Project title: Self-sufficient Integrated Multi-Trophic AquaPonic systems for improving food production sustainability and brackish water use and recycling

Periodic Technical Report

Part B

Period covered by the report: from [01 June 2019] to [30 November 2020]

Periodic report: [1st]

Contents

1. Explanation of the work carried out by the beneficiaries and overview of the progress

The international project entitled "Self-sufficient Integrated Multi-Trophic AquaPonic systems for improving food production sustainability and brackish water use and recycling - SIMTAP" was approved in November 2018 as part of the PRIMA (Partnership for Research and Innovation in the Mediterranean Area) call 2018 (Section 2) and started on 1 June 2019.

The project is coordinated by Prof. Alberto Pardossi of the University of Pisa (UNIPI), which participates in the project with two departments: the Department of Agricultural Sciences , Food and Environmental (DiSAAA), to which Prof. Pardossi belongs, and the Department of Veterinary Sciences (DiSVe), to which Prof. Carlo Bibbiani and Dr. Baldassare Fronte belong.

Other partners are the following:

- University of Bologna, Department of Agro-Food Sciences and Technologies, Bologna (Italy) (UNIBO; team leader: Prof. Daniele Torreggiani)
- University of Milan, Department of Environmental Sciences and Policies, Milan (UNIMI; team leader: Dr. Jacopo Bacenetti)
- INRAE-Agrocampus, SAS Sol Agro et hydrosystème Spatialisation, Rennes (France) (INRAE; team leader: Dr. Joel Aubin)
- Lycée de la Mer et du Littoral, Bourcefranc le Chapus (France) (LML; team leader: Dr. Vincent Gayet)
- Mediterranean Fisheries Research Production and Training Institute, Antalya (Turkey) (MEDFRI; team leader: Dr. Mehmet Ali Turan Koçer)
- Ministry for Agriculture, Fisheries and Animal Rights, Agriculture Directorate Marsa (Malta) (MAFA; team leader: Marcelle Agius)
- Korolev GmbH, Bonn (Germany) (KOROLEV; team leader: Dr. Rainer Linke)

In the proposal, the partner in Malta was Ministry for the Environment, Sustainable Development & Climate Change, and the team leader was Mr. Kyle Spiteri. Due to the reorganization of the Ministry, the name was changed in 2020.

The project obtained funding of approximately 953,000 euros. Unfortunately, although the project officially started on June 1, 2019 and the kick-off meeting was held on June 26-27 in Pisa, due to the long and complex procedure provided by the Italian Ministry of University and Research (MUR) for the funding of international research projects, the grant agreement (named Atto d’Obbligo, in Italian) between MUR and the three Italian universities has been not signed yet. Consequently, no funding was distributed by MUR and only UNIPI and UNIBO received a partial advance of the funds from their own university. Regarding UNIMI, no funds were given from the Italian Ministry as well as no fund anticipation was granted by the University. Consequently, until now, the expenses for the participation to the kick-off meeting as well as for the technical visits carried out in aquaculture farms (total amount about 5500 €) were anticipated using, temporarily, other funds of the research group.

1.1 Objectives

Tab. 1. Work performed and results achieved for the projects objectives as described in section 1.1 of the proposal

Objective	WP	Key performance indicator (KP1)	Work performed and results
SO1 - Designing a SIMTAP system with integrated smart monitoring and control system (ISMaCS) The project aims at designing SIMTAP systems to be implemented in different contexts in Mediterranean areas. The technological specifications of every component of the systems will be identified and reported in a document.	1	Executive project documents and technical specifications	The designs of the four SIMTAP systems set up in France, Italy, Malta and Turkey the smart monitoring were completed in due time.
SO2 – Development of four SIMTAP prototypes in different geographic contexts Four prototypes will be built in different Mediterranean countries (Italy, Turkey, France, and Malta) with diversified environmental and salinity conditions. Various combinations of inputs will be considered in order to optimize biomass production based on local specific features, opportunities and constraints	2	Set-up of the prototypes	Three SIMTAP systems were set up in France (INRAE, LML), Turkey (MEDFRI) and Italy (UNIPI, UNIBO) and were used for experiments in 2020. The construction of the system in Malta (MAFA) is still in progress.
SO3 - Evaluation of effectiveness, efficiency and performance of SIMTAP systems. The prototypes will be evaluated in terms of biomass, food and feed production, and waste production in comparison with conventional aquaculture and hydroponic systems	2	Food and feed productivity of the prototypes in comparison with currently available aquaculture and hydroponic systems	Results on fish growth performance at early and fattening stage in SIMTAP system are already or will be soon available (INRAE, LML, MEDFRI, UNIPI). Results on growth performance at fish early stage together with halophyte plant, microalgae, polychaetes as well as nutrient mass balance estimation in the SIMPTA systems installed in several countries will be carried out in 2021.

Objective	WP	Key performance indicator (KP1)	Work performed and results
SO4 - Development of Decision Support System (DSS) for SIMTAP implementation DSS aimed at defining the optimal locations of SIMTAP systems in the different contexts will be developed in GIS environment with a multi-criteria approach. Moreover, in order to conduct a sustainability assessment, aggregating the different indicators proposed by the different methods (LCA, LCC, Emergy), a qualitative approach based on decision trees will be carried out, applying a decision support system software stemmed from DEX methodology	3-5	GIS tool to assess SIMTAP location. DEXi tool	INRAE developed the Dexi methodology adapted to SIMTAP systems and built the tools for data collection and calculation of indicators, and the structure of the decision trees.
SO5 – Quality assessment of food products The objective is to assess the physical and chemical quality of food produced within the SIMTAP systems.	4	Chemical parameters of food products	Quality of crop plants grown in the SIMTAP prototype installed in Italy was assessed in a series of experiment conducted in 2020 by UNIPI. MEDFRI’s studies on quality of fish and crop produced in SIMTAP system will be completed following the production in next period of the project.
SO6 - LCA of SIMTA. Life Cycle Assessment (LCA) will be carried out in order to assess environmental impacts from each SIMTAP system. Different parameters will be determined per unit of food produced to describe the use of resources and the environmental impacts.	5	LCA parameters	A data collection tool was develop by INRAE to collect the useful data for life cycle inventory.
SO7 - Economic assessment of SIMTAP. Life Cycle Cost (LCC) will be carried out in order to assess the economic performances of each SIMTAP system under investigation. LCC analysis	5	Cost and investment analysis	INRAE and UNIMI developed a form to collect economic data about production factors and the capital goods and revenues SIMTAP systems.

Objective	WP	Key performance indicator (KP1)	Work performed and results
will provide a detailed account of the total costs of a SIMTAP system over its expected life. European subsidy framework will be also investigated.			An Excel-based tool for the calculation of the economic performances of the different SIMTAP systems was developed by UNIMI.
SO8 - Emergy accounting of SIMTAP. Emergy accounting (EA) will be carried out in order to complete the environmental assessment of each studied SIMTAP system.	5	Emergy parameters and indicators.	A tool was develop by INRAE to collect the useful data for Emergy accounting adapted to SIMTAP systems.
SO 9 - Social assessment of SIMTAP. Social Life Cycle Assessment (SLCA) will be carried out in order to assess the social performances from each SIMTAP system under investigation. Social indicators will be determined such as working hours, creation of new jobs and working opportunities, involvement of local population, improved working conditions.	5	Social indicators	INRAE and UNIMI developed **a** form to collect data to assess social sustainability of SIMTAP systems.
SO10 - Context of circular economy. The project output will be disseminated among the scientific community, economic operators and other stakeholders. Specific recommendations, guidelines, best practices, and training activities will be designed and developed in order to boost the exploitation of SIMTAP systems in the Mediterranean areas with the aim to create technical skills and job opportunities, and update trading system.	6-7	Dissemination events, technical documents (recommendations, guidelines, handbooks), training platforms and activities	Main results: project website; Youtube video of SIMTAP system in France; scientific publications.

1.2.1 Work Package 1

Title: Ecosystem based approach for SIMTAP

WP Leader: UNIPI

WP Participants: ALL

Start month: 01 **End month: 06** (the end of this WP was postponed to M21)

Objectives: WP1 aims to: i) to design and build the SIMTAP prototypes and their monitoring and control systems in France, Italy, Malta and Turkey according to local constraints and the mutual interaction of each unit (aquaculture, hydroponic, photobioreactors for algae, filter/feeder modules etc.); ii) to identify on the basis of scientific and technical literature and project partners' experience: the most suitable**.** hydroponic systems; the most suitable species of crop plant, algae, fish, polychaetes and other detritivore and filter feeder organisms (DFFO) for the SIMTAP system; the level of inclusion of DFFO, in place of fishmeal and fish oil, in the diet of fish reared in the SIMTAP systems.

Nine deliverables are foreseen for this WP, including the pilot systems.

Task 1.1 Piloting activity: Designing, building and first start of SIMTAP prototypes or specific sections of it, for their use under different Mediterranean climatic conditions, starting from the concept of the Patent Gebrauchsmusterschrift DE 20 2014 103 397 U1 2015.12.03 (M1-M6). Task leader: UNIPI /Partners involved: INRA, LML, MEDFRI, MESDC.

Work performed and main results

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 circulation, and thus the same environment, 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) is currently 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 30 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.

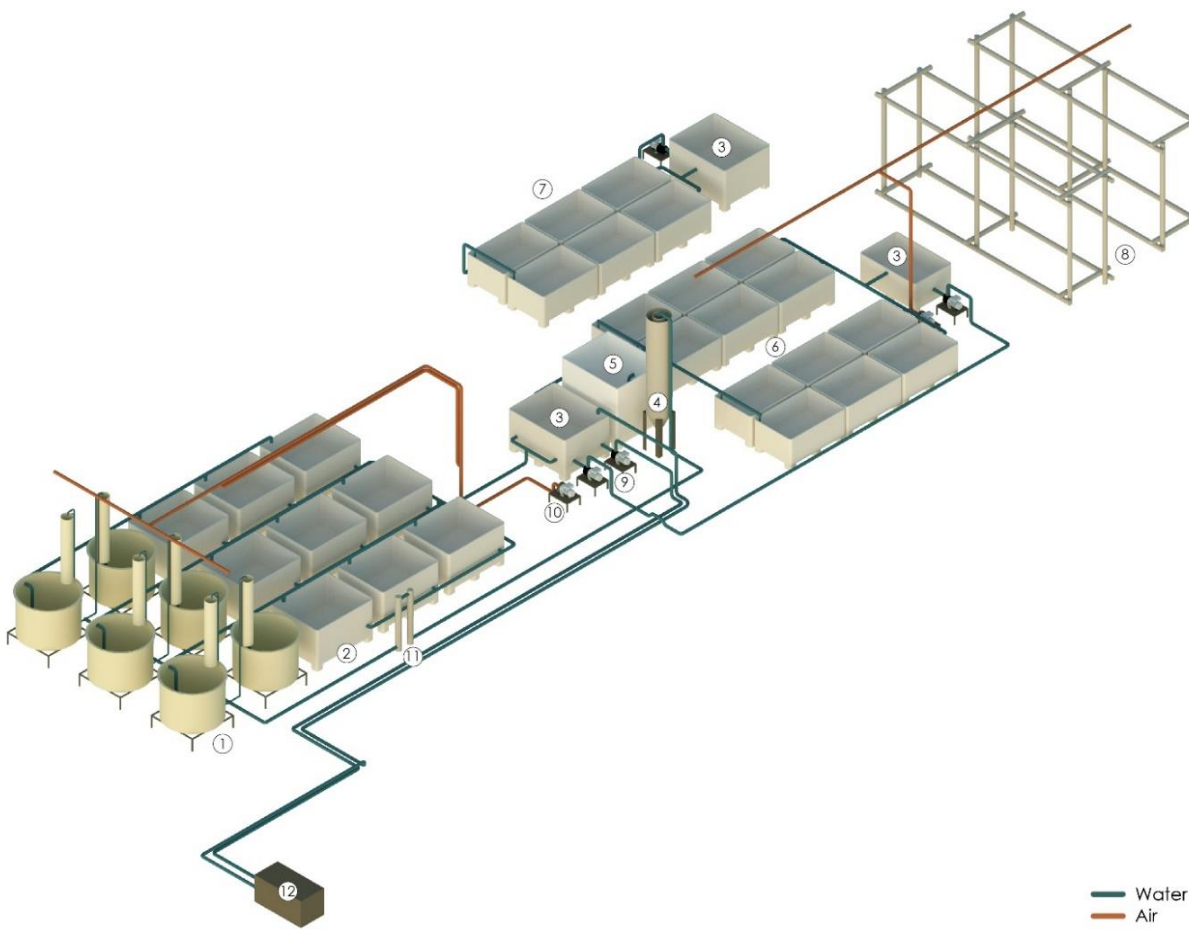

Fig. 1. 3D scheme of SIMTAP at UNIPI. Different components of the system are listed: (1) fish rearing tank, (2) FFDO tanks, (3) sump, (4) settler, (5) biofilter, (6) hydroponic and macroalgae section, (7) brackish water section, (8) microalgae section, (9) pumps, (10) blower, (11) UV steriliser, (12) 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 30 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 DWC hydroponic 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 centimeter 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 temperature (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) in order 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.

DDFO laboratory

An experimental facility was built in the spring 2020 at the University of Pisa for laboratory-scale experiments with DDFO (**Fig. 3-4**). This system allows to test different treatments (up to 6 treatments with 3 replicates each) contemporaneously. In 2020, the laboratory was used for experiments on breeding and feeding of polychaetes.

The system is basically a closed loop, constituted of:

1. A two floor Zn-coated steel shelving unit
2. Recirculating 77-L (0.40 x 0.60 x 0.32 m) plastic tank (sump) with an electric heater (600W) connected with an external thermostat and a timer-controlled submerged pump (0.25 kW, 0.33 Hp).
3. Filter system constituted of a double stage filter (0.25 m, 100 µm mesh)
4. Disinfection unit with UV lamp (18 W);

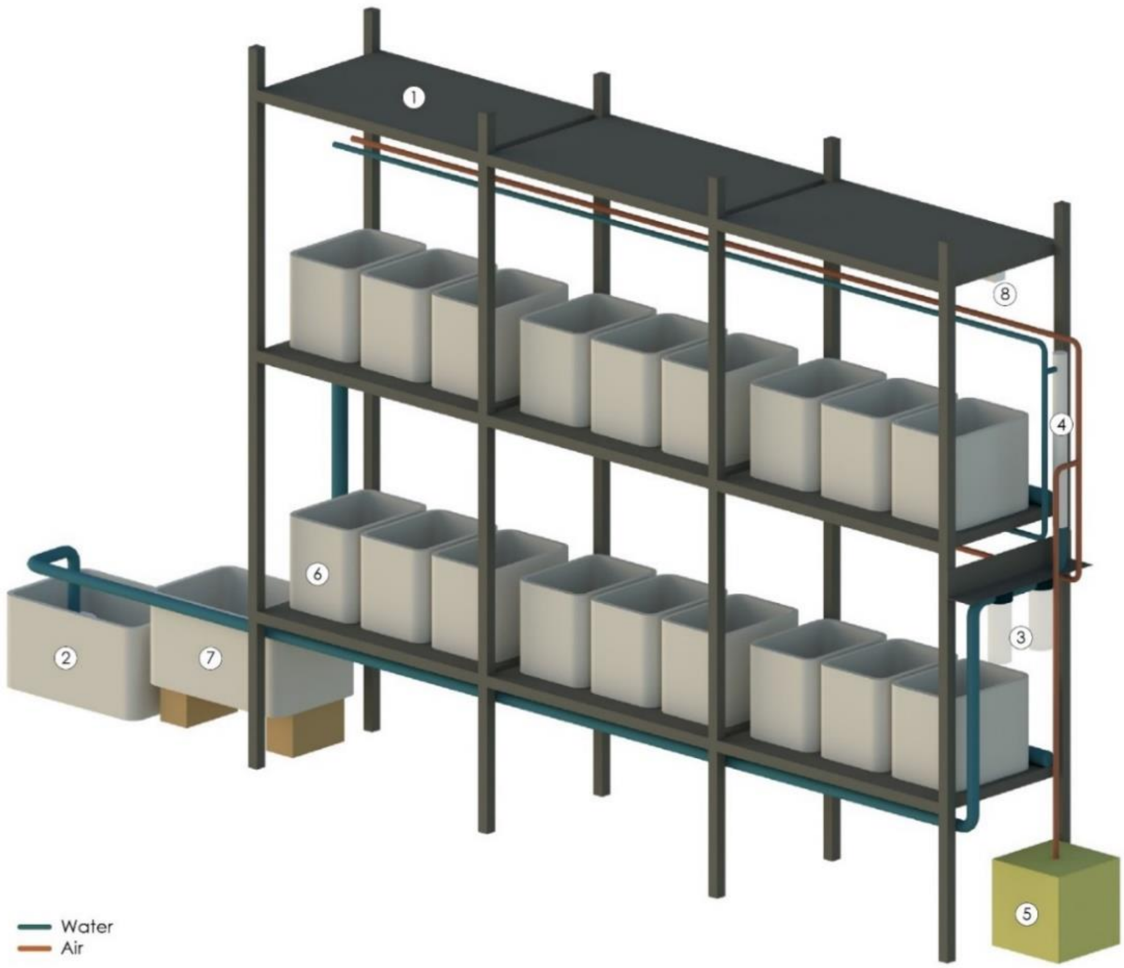

Fig. 3. 3D sketch of the layout of the facility for experiments with polychaetes: shelf (1); recirculating tank (2), filters (3), UV lamp (4), air compressor (5), rearing tanks (6), biofilter (7), LED lamp (8).

5. Timer-controlled silent air compressor (8 bar, 6 L, 59 db) controlled by a digital timer
6. 18 rearing tanks 38-L (0.40 x 0.30 x 0.32 m) plastic (HDPE) tanks;
7. Biofilter constituted of a 77-L plastic tank with cubic sponges to support bacteria growth
8. Timer-controlled LED lighting system (30 W, 120 lumen W^{-1}) providing luminous flux of 150 lumen at the water surface.
9. Hydraulic lines consisting of a plastic (PVC) pipe (3/4 inch) that connect in series: pump, filters and UV lamp. Each tank in the system is supplied from a 5 mm pipe with a valve. The outlet pipe is constituted by a PVC pipe (2 inches) placed behind the rearing tanks, which collect water from the overflow hole of the rearing tanks. The water flow is (Fig. 1) is: sump > filter > UV lamp > rearing tanks > biofilter > sump. The submerged pump moves the water up to the rearing tanks, then it comes back in the sump, through biofilter, by gravity.

10. The air line consisting of 0.2 mm PE pipes for each shelf. These are connected directly to the air compressor through a pneumatic valve that controls the air flow in the rearing tanks. Air is dispensed into each tank through a porous pipe connected with the main tube with 5 mm pipes flow (Fig. 1) is: air compressor > pneumatic valve > rearing tanks.

The laboratory is also equipped with two refrigerators (2-8 °C, volume 244 L) with LED stripes and air supply system; they are used to induce thermal shock to some DFFOs in order to stimulate their reproduction.

Fig. 4. Some views of the laboratory used for the experiments with DFFO: shelving unit and rearing tanks with aerators.

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**. It 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 in order 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.

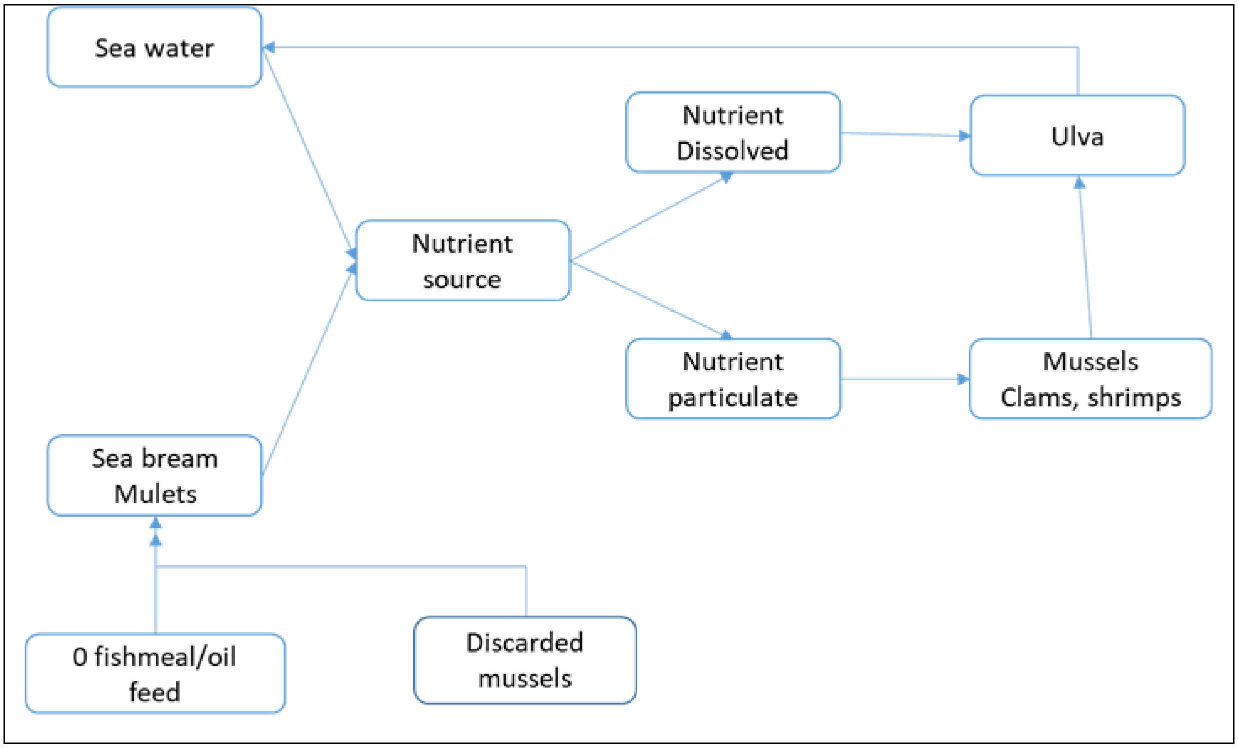

Fig. 5. Representation of principles of LML-INRA case study.

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.

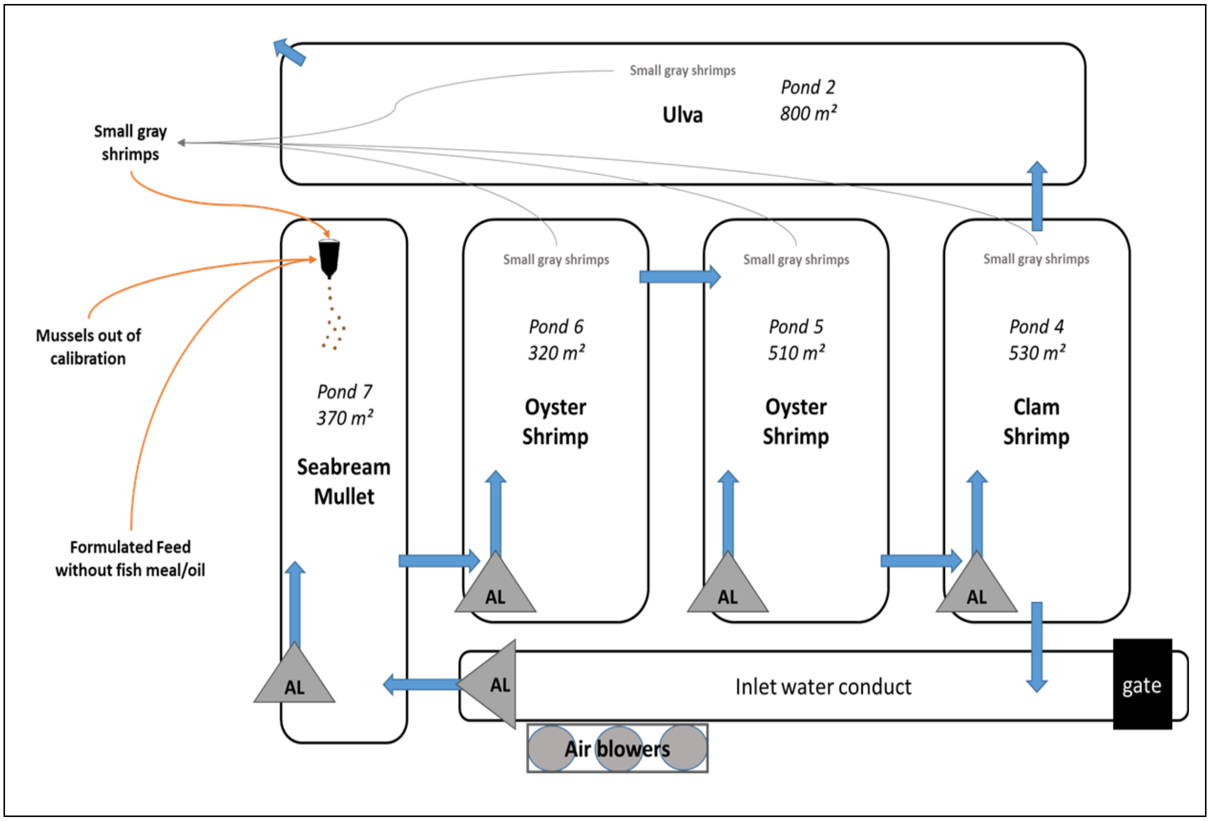

Fig. 6. Diagram of the experimental design of the SIMTAP system at INRAE-LML

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, as a consequence, 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**).

Description of 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 deposit feeder 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).

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 is supplied from the Mediterranean Sea with an average salinity of 38 ppt. Water losses resulting from evaporation are renewed with a mixture of marine and brackish water to avoid a salinity increase.

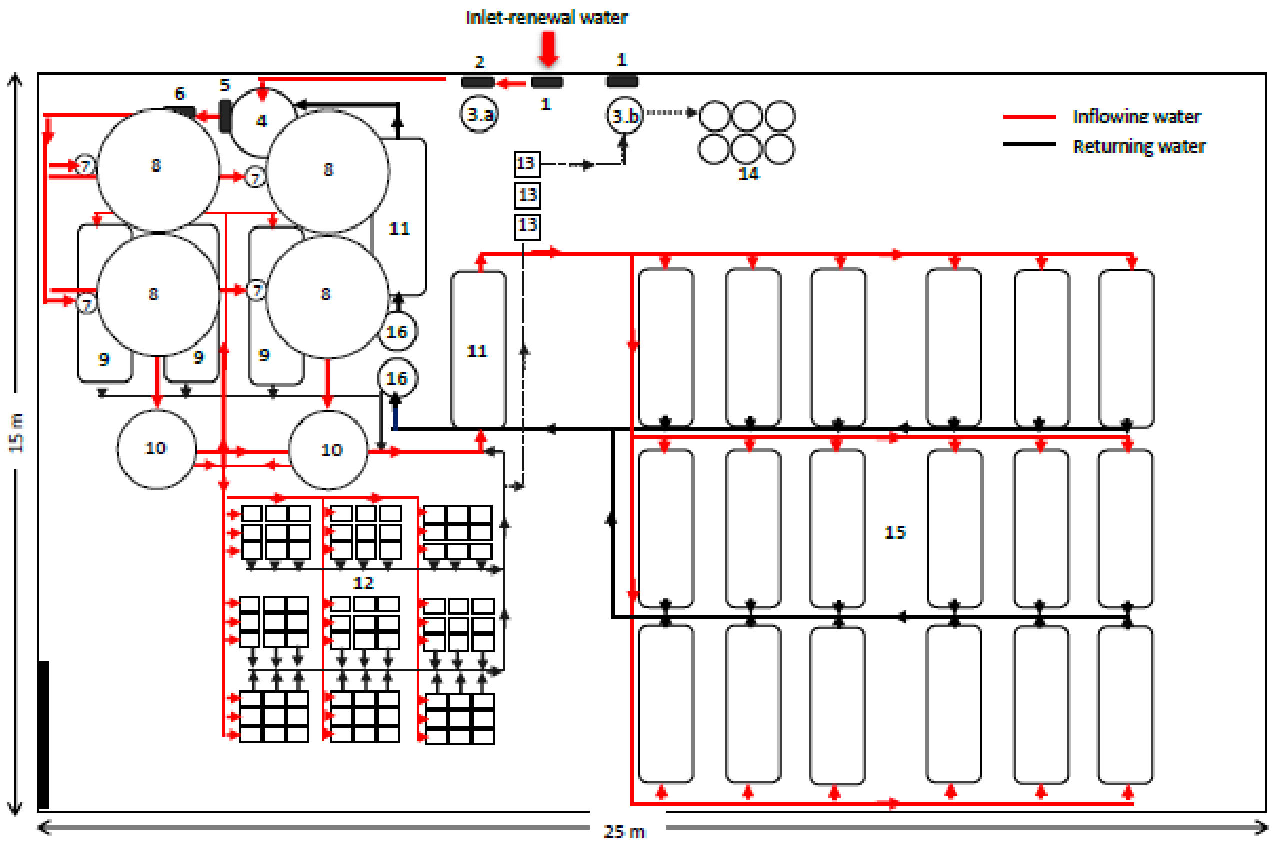

Fig. 7. Process flow diagram for SIMTAP system at MEDFRI (1. Particle filter with a capability of 10 µm, 2. Ozone generator (2 g per sec), 3. Ozonation tank (a-0.5 m^3; b-0.1 m^3), 4. Sump tank (3 m^3), 5. Water pump (a speed and flow controlled heavy duty pump; 0.75 kW), 6. UV lamp (58 W; 846 mm lamp 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)).

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 aquaculture hatcheries.

Halophyte unit: Halophyte growing unit based on the Deep Water Culture (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 (due to results, 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 galbana*, *Nannochloropsis oculata*, *Chlorella vulgaris* etc.) will be tested. The strains will be supplied from the microalgae culture collection of MEDFRI.

Deposit feeder 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* that will be collected from natural populations of the coastal area of the Aegean Sea will be 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 deposit/filter feeder 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 Deep-Water Culture (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.

Task 1.2 Theoretical study of the most suitable plants (edible and nutraceutical/pharmaceutical), algae, and live organisms to be integrated in the SIMTAP system (M1-M6) Task leader: UNIPI /Partners involved: INRA, MEDFRI.

UNIPI

Algae: Green microalgae and macroalgae (seaweeds), which will be cultured, respectively, in photobioreactors or hydroponic-like tanks in the SIMTAP prototype installed in Italy, were identified on the basis of on current literature and locally available organisms. Microalgae will be grown for feeding polichaetes while macroalgae will be grown to remove inorganic nitrogen from the effluents of the aquaculture sector.

Many microalgal strains might be appropriate for polychaetes feeding, e.e. *Dunaliella* sp., *Isochrysis* sp. *Chlorella vulgaris*, *Chlorella sorochiniana*, *Nannochloropsis oculata* and *Tetraselmis chuii*. Selected seaweed species were *Ulva rigida* C. Ag. and *Chetomorpha linum* (Müller) Kützingas. These species can be easily collected along the coast nearby Pisa, in particular in the shallow lagoon in Orbetello, where effluents from land-based aquaculture farms are discharged.

Plants

DEXi multi-criteria analysis was adopted for the selection of suitable plant species for SIMTAP system. The DEXi analysis is a relatively easy and effective tool, which can be used for the selection of proper plant species in similar contexts.

In the first instance, a survey was carried out with different researchers with expertise in the field to select the most important criteria for the evaluation. One simple tree structure which contained one root criterion ('Plant species selection'), four aggregated criteria, and four sub-criteria was created as a result of the survey. The weights assigned to the aggregated criteria, sub-criteria, and indicators were selected by a panel of experts and concerning the objectives set for the SIMTAP project. Afterwards, an extensive literature analysis was conducted to identify the potential species and their features for the evaluation.

Provided that suitable fish species for the SIMTAP system are euryhaline and can grow properly in a wide range of salinity, the same model was evaluated under two different scenarios (two different salinity levels): 35 and 10 g/L. Finally, the relevance of the model structure was evaluated by the sensitivity analysis, through the ‘plus/minus-1’ analysis. Fifteen salt-tolerant plant species were selected for the analysis.

The study suggested that *S. europaea* L. and *P. oleracea* L. were the most suitable species for the SIMTAP system with a water salinity of 35 g L^{-1} while at lower salinity level (10 g/L) the best candidate species were five: *S. bigelovii Torr*, *S. europaea L.*, *B. vulgaris* ssp. *Maritima* (L.) *Arcang*, *A. hortensis L.*, and *P. oleracea L.* The ‘plus-minus 1’ analysis confirmed that the final scores were influenced by the weights assigned to each aggregated criterion.

Fish

In Integrated Multi-Trophic Aquaculture (IMTA), two kinds of organisms coexist: some organisms (fish, f.i.) generate produce wastes (uneaten feed, faeces etc.) that the extractive organisms convert into fertilizers, food and energy. Similarly, SIMTAP is a multi-trophic system where extractive species are crop plants (salt-tolerant glycophytes or halophytes), while in a marine IMTA, shellfish and macroalgae are commonly used. The peculiar rearing environment of SIMTAP, drastically influences the generative and extractive species exploitable: the use of saline water (seawater or brackish water, including greenhouse’s runoffs), the high stocking densities in fish section and reduced water renewal, and the possible interactions within different trophic levels. Thus, the biological characteristics of fish and other species to be cultured in SIMTAP systems should be assessed taking into account their complementarity and adaptability to the physical and technical traits of the considered system.

The study aimed to identify the most suitable marine organisms for food production in the SIMTAP system under the typical environmental and market conditions of the Mediterranean area through the creation of a decision model (DEXi_SIMTAP_Fish_1.0). A panel of experts of the University of Pisa, representatives of the main scientific sectors involved in the SIMTAP project (engineering, aquaculture, aquaponics, hydroponics, marine biology and animal nutrition) was selected in order to define and implement the decision model. Firstly, a comprehensive analysis of the available literature was carried out on fish species considered as relevant for the Italian SIMTAP production, the species selected were: Sturgeon (*Acipenser* spp., ST), Meagre (*Argyrosomus regius*, ME), European Sea Bass (*Dicentrarchus labrax*, ESB), Sharpsnut Sea Bream (*Diplodus puntazzo*, SSB), Mullet (*Mugil cephalus*, FGM), Common Octopus (*Octopus vulgaris*, CO), Turbot (*Psetta maxima*, TU), Greater Amberjack (*Seriola dumerili*, GA), Sole (*Solea spp*, SO), Gilthead Sea Bream (*Sparus aurata*, GSB), Shi Drum (*Umbrina cirrosa*, SD).

Secondly, based on collected data, the experts performed the SWOT analysis for identifying the most relevant species-related attributes to be considered in the decision process.

The decision model was created using DEXi software. To this regard, a “top-down” approach was used in order to identify criteria and indicators (arising from attributes identified by SWOT analysis) that compose the decision tree. Utility function rules were created using the “weight editor” function of the software, weights (expressed as a percentage) were discussed among the members of the panel that eventually voted to make the final decision. The tree structure (**Fig. 8 and Tab. 3**) consisted of one root criterion (“Fish species selection”) branched into 2 not-aggregated and 4 aggregated criteria (2 indicators each): “geo distribution”, “domestication” (aggregated from “farm know how” and “hatchery availability”), “environmental needs” (aggregated from “salinity” and “temperature”), “feeding regime” (aggregated from “wild feeding behaviour” and “nutritional knowledge”), “growth performances” and “market value” (aggregated from “raw products” and “processed by-products”). Short value scales (from 2 to 5 values) were selected to reduce the number of decision rules for the aggregated criteria, thereby avoiding its “combinatory explosion. Finally, a sensitivity analysis (“plus-minus 1”) was performed. All these species can be naturally found in the Mediterranean Sea; this was a starting point of the selection process. Thus, the “Geo-distribution” criterium was considered less influent in the decision process.

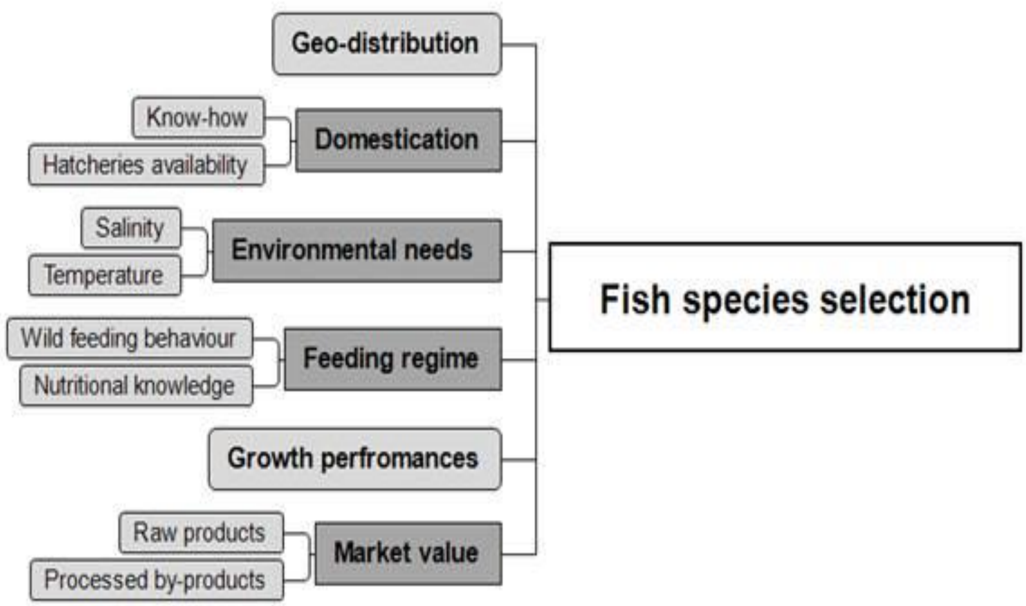

Fig. 8. Tree of attributes. Boxes with bolded letter represent criteria; blue boxes represent indicators or not-aggregated criteria; grey boxes represent aggregated criteria.

Regarding the economic criteria, GSB and ESB are consolidated species characterized by relevant market value. GA, TU, SO and CO are well-appreciated species on the market, even though their farm production is quantitatively limited. The raw products of FGM and ST are hardly appreciated by consumers and their market price is very low, however, the production of processed by-product such as salted mullet roe ("bottarga") and caviar may represent a great opportunity for SIMTAP production. Nevertheless, the market value was considered less important in the decision process due to the main goal of the SIMTAP project: the implementation of the ecosystem concept in the management of aquaponic systems.

Tab. 3. Results obtained from the DEXi_SIMTAP_Fish_1.0 decision model for the "Fish species selection" and aggregated criteria (according to the decision rules) and value assigned to each indicator by the panel of experts. The results of sensitivity analysis is shown by the asterisk.

Criteria & indicators	%	Species										
		GSB	**ESB**	**FGM**	**SSB**	**GA**	**SD**	**ME**	**TU**	**ST**	**SO**	**CO**
Geo-distribution	4	H	H	H	H	H	H	H	H*	H*	H	H
Domestication	**24**	**H**	**H**	**M**	**L**	**L**	**M**	**M**	**M**	**M**	**M**	**L**
└ Know-how	25	H	H	H*	L*	L	H	H	H*	H	H	L
└ Hatcheries availability	75	H*	H*	L	L*	L*	L*	L*	L	L*	L*	L*
Environmental needs	**24**	**H**	**H**	**H**	**H**	**M**	**H**	**H**	**H**	**L**	**H**	**L**
└ Salinity	50	H*	H*	H*	H	L*	H	H	H*	L*	H	L
└ Temperature	50	H*	H*	H*	H	H*	H	H	H*	L*	H	L
Feeding regime	**24**	**H**	**H**	**M**	**H**	**M**	**H**	**H**	**M**	**M**	**H**	**L**
└ Wild feeding behaviour	25	H	L	H*	H	H*	H	H	H*	H	H	L
└ Nutritional knowledge	75	H*	H*	L	H	L	H	H	L	H	H	L*
Growth performances	20	L	L	H*	L*	H*	H	H	L	L*	L*	H*
Market value	**5**	**H**	**H**	**M**	**L**	**H**	**L**	**L**	**H**	**M**	**H**	**H**
└ Raw products	75	H*	H*	L	L*	H	L*	L*	H*	L*	H	H*
└ Processed by-products	25	L	L	H	L	L	L	L	L	H*	L	L
Fish species selection		**Excellent**	**Excellent**	**Good**	**Medium**	**Medium**	**Good**	**Good**	**Good**	**Poor**	**Good**	**Poor**

Legend: 'GSB' Gilthead seabream; 'ESB' European seabass; 'FGM' Flathead grey mullet; 'SSB' Sharpsnut seabream; 'GA' Greater Amberjack; 'SD' Shi Drum; 'ME' Meagre; 'TU' Turbot; 'ST' Sturgeon; 'SO' Solea; 'CO' Common Octopus; 'H' High; 'M' Medium; 'L' Low; Bold letter: indicates aggregated criteria; Grey highlighted rows indicates criteria; Asterisk '*' means that a 'plus-minus 1' change of the indicator's score affects the 'Final score';

The choice of fish species for an aquaculture system depends principally by the salinity and the temperature of the water used. In the SIMTAP prototype installed in Italy, the salinity of the system could reach 35 g L^{-1} and temperature could range between 18 and 25 °C, which are optimal values for most of the considered species. For these reasons, euryhaline and eurythermal features of the candidate species were taken under high consideration. Under these perspectives, most of the considered species were adequate for the SIMTAP conditions. The stenohaline species GA and CO, as well as ST, which needs freshwater and low temperature, were penalized.

Due to the high relevance of the sustainability issues within the SIMTAP concept, the feeding regime was highly considered by the panel of experts and split into two different aspects: the "wild feeding behaviour" and the "nutritional knowledge" so far available, the latter to ensure a successful rearing activity. In detail, the adaptability of the species to be fed on different sources, namely alternatives to fish meal (FM) and fish oil (FO), in addition to their natural attitude (carnivorous or omnivorous) was thoroughly analysed. In fact, the SIMTAP concept takes under consideration the use of self-produced marine detritivores and filter-feeder organisms (e.g. echinoderms, polychaetes and mussels) for replacing FM and FO. FGM and SSB, which can feed on a wide variety of substrates thanks to their omnivorous habit. SO naturally feed on polychaetes and other benthic organisms.

Moreover, Kals et al., (2017) reported that the use of ragworm-extract (*Nereis virens* Sars) in the diet of adult SO alleviates the anaemia and positively affected the SO metabolic performance (feed intake, feed efficiency and growth). Carnivorous species such as ESB, GSB, SD, ME and ST are nowadays successfully fed on feeds rich in ingredients alternatives to FM and FO, mainly plant products and by-products. Exceptions are TU, since its diet is still based on fish raw material, and GA and CO whose optimal feeding regime is not well known. FGM nutrition is largely based on the exploitation of natural food sources in extensive farming systems such as lagoons and limited knowledge is available on the nutritional requirement of this species.

The territorial distribution and availability of hatcheries were considered as strengths since it may facilitate the juvenile supply for SIMTAP production and favourable juvenile market price. Under this perspective, GSB and ESB juveniles are largely available on the Mediterranean area and notably in Italy. The other species suffer other limits such as: the re-creation of optimal environmental conditions within the hatcheries (e.g. tanks sizes and shapes), the need of hormonal treatments to induce spawning, and the lack of well-known farming techniques. Nowadays, hatcheries production of SSB, GA, SD, ME and TU is absent or very limited in other countries, such as Spain for ME and TU. The reproduction of FGM requires hormonal treatments that increase the running costs, and therefore the hatchery production of FGM juveniles has never reached a significant commercial scale. The farming know-how was also considered as a relevant indicator. Under this perspective, GSB and ESB are the most reared fish species in the Mediterranean area and the farming know-how and domestication level have already reached very high standards. In contrast, species such as GA and CO are not largely reared yet due to several bottlenecks in their farming process.

Regarding the growth performances of the candidate species, fast-growing species such as FGM, GA, SD, ME and CO were the best candidates for SIMTAP production. TU growth performances were also relevant, on the contrary, GSB and ESB showed a reduced growth rate in comparison with the other species of the group.

The output of the model was the final score for the selected fish species (**Tab. 1**). GSB and ESB were scored as “excellent” and appeared the most suitable fish species for the SIMTAP system. Strengths of these two species were the domestication level already reached, the farming know-how and, in particular, the deep knowledge of nutritional requirements and feeding, and the market value. Other species were scored “good”: MU, SD, ME, TU and SO. They are alternative to GSB and ESB.

Detritivore and filter feeding organisms (DFFO)

A thorough literature survey was done to select the most appropriate detritivore and filter feeding organisms (DFFO) to be incorporated in the SIMTAP system. Main emphasis was given to locally available species to minimize the risk associated with introduced species. Three groups of DFFO were selected *viz*.: Polychaetes, Bivalves and Holothurians. Several families of polychaetes (mostly in the families *Arenicolidae*, *Eunicidae*, *Glyceridae*, *Lumbrineridae*, *Nephtyidae*, and mainly *Nereididae*), are gaining popularity very rapidly owing to its commercial importance.

Among the polychaete families we decided to focus on the *Nerididae* family and, especially on *Hediste diversicolor*, owing to its many advantages it has over other species, especially with respect to *Nereis virens*. *H. diversicolor* is a geographically widely distributed inhabitant of the intertidal zone of marine and brackish waters. It can be found as far north as Scandinavia and as far south as Morocco, in the cold and brackish Baltic Sea as well as in the hot and hypersaline lagoons of the Black Sea. It is known to tolerate a wide range of salinity down to 2-3‰ (euryhaline), whilst, *N. virens* is rarely found below 15‰ salinity (Jørgensen and Dales, 1957). However, the most conspicuous feature of *H. diversicolor* is the unique ability to obtain nourishment as a facultative filter-feeder (Riisgård 1991, Riisgård et al. 1992; Vedel and Riisgård 1993; Vedel et al.1994). In fact, *H. diversicolor* utilizes a variety of feeding strategies: i) as filter feeder feeding on diatoms and other micro-organisms (Harley 1950; 1953); ii) as deposit feeder feeding on sediments and particulate organic matters (Reise 1979; Cram and Evans, 1980); iii) as a scavenger feeding on the dead decayed matter (Olafsson and Parsson, 1986; Heip and Herman, 1979); iv) predator feeding on small meiobenthic organisms (Reise 1979; Witte and Wilde, 1979; Ratcliffe et al. 1981; Rönn et al. 1988). Moreover, on the reproductive front, *H. diversicolor* is typically atoquous *i.e.* the change of body form preliminarily to spawning resulting to a free-swimming epitoquous or ‘heteronereid’ form is absent (Dales 1950). Another important factor considering the breeding and production of Nereids is the sediment type that will provide shelter and act as breeding ground for these worms. *Hediste diversicolor* is known to tolerate in a wide range of sediment type and it was demonstrated that the size range of sediment tested (medium: 0.5-0.25mm and fine: 0.25-0.125mm), did not cause differential growth of *H. diversicolor* (Fidalgo e Costa 1999).

From amongst the available study on bivalves, we have decided that the most suitable species for the SIMTAP system to be *Mytilus galloprovincialis* and *Tapes philippinarium*. *M. galloprovincialis* is a native species of the Mediterranean coast and the Black and Adriatic Seas, though it considered as an invasive species outside its geographical range. Better growth, higher survival rates and faster metamorphosis were observed in *M. galloprovincialis* larvae cultured at 20°C or 24°C (Sánchez-Lazo and Martínez-Pita 2012) and a favourable salinity within the range of 27 – 40 ppt (https://www.cabi.org/isc/datasheet/73756#tosummaryOfInvasiveness). *R. philippinarum* is euryhaline. The spat are able to tolerate a wide range of salinity ranging from 14 – 33.5 ppt, with 20.5 ppt as the optimum (Lin et al. 1983). In addition, they demonstrate a high salinity tolerance (7.5 – 40 ppt) which is the basis for the clam's successful establishment in the estuarine conditions.

Sea cucumbers are considered important processors of surface sediments in many coastal marine systems as they play an important role in sustaining the health of marine ecosystems (Purcell 2010). Apart from nutrients redistribution sea cucumbers also play an active role on ocean acidification buffering, due to ingestion and release of fecal matter and by their locomotion across the sea bed. Some species can facilitate calcification by organisms such as corals by increasing water alkalinity and dissolved inorganic carbon through their digestive processes and release of ammonia. Selected sea cucumber species were *Holothuria tubulosa* and *H. polii*. They are among the most common sea cucumbers species found in the Mediterranean Sea (Tortonese 1965).

Task 1.3 Theoretical study of the dietary inclusion levels of polychaetes and of other deposit/filter feeders, in place of fishmeal and fish oil, in diets of European sea bass and sea bream, mullets, etc., in order to determine the substitution level that can be safely adopted in SIMTAP applications without fish growth and quality deteriorations. Theoretical study of the diets of polychaetes and other deposit/filter feeders (M1-M6). Task leader. UNIPI/Partners involved: INRA, MEDFRI, MESDC.

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Polychaetes

Polychaetes are excellent scavengers and detritus consumers. Their activity turns over the upper layers of sand beds, working detritus into the sand, thereby preventing clumping. The utilization of organic extractive culture (e.g. suspension feeders/deposit feeders) in integrated systems has proven to be a valid alternative for nutrient bioremediation. Polychaete worms also have been identified as excellent sources of protein and n-3 long chain poly-unsaturated fatty acids which play an essential role in aquafeeds. Moreover, it plays an important role in stimulating gonad development and spawning in several cultured species (Fidalgo e Costa et al. 2003). The potential of polychaetes to effectively consume aquaculture production waste and convert them into valuable compounds as marine proteins and lipids has been widely studied (**Tab. 4**).

Tab. 4. List of selected polychaete species studied for aquaculture waste bioremediation

Species	Co-culture/fed with	Reference
Sabella spallanzanii	Mediterranean mussel	Giangrande et al. 2014
Perinereis nuntia vallata	Japanese flounder	Honda and Kikuchi, 2002
Perinereis nuntia	Prawn farm waste	Palmer 2010
Perinereis helleri		
Nereis virens	Atlantic Halibut waste	Brown et al. 2011
Hediste diversicolor	Mysid crustaceans	Bradshaw et al. 1990
Hediste diversicolor	Euphausiids and copepods	Uttal and Buck, 1996
Hediste diversicolor	Plant materials	Olivier et al. 1996
Hediste diversicolor	Carpet shell clam	Batista et al. 2003
Hediste diversicolor	Eel sludge	Garcia-Alonso et al. 2008
Hediste diversicolor	Sea Bream waste	Bischoff et al. 2009
Hediste diversicolor	European sturgeon	Pajand et al. 2017
Hediste diversicolor	Effluent water fish farm	Marques et al. 2018
Hediste diversicolor	Salmon smolt waste	Wang et al. 2019

The remediation efficiency of farms organically enriched by aquaculture was enhanced by the introduction of polychaetes. This may be accounted either by the direct consumption of the waste by the worms or by bioturbation activity (Bergstorm et al. 2015). Several studies indicate that the nutritional compositions of the worms' tissues are proportional to the feed given. More importance is given on the fatty acids and protein profile as they are of great importance for the food webs and are in high demand for both human and animal nutrition (Bischoff et al., 2009). Amongst the polychaete species, *Hediste diversicolor* reared on wastes from land-based aquaculture systems has a high potential for intensive production owing to its coprophagous feeding behaviour (Wang et al. 2019). These findings suggest that *H. diversicolor* can ingest as well as recycle huge amounts of aquaculture generated sludge, whilst maintaining proper fatty acid levels when compared to those worms fed on commercial fish feed (Wang et al. 2019). This supported the view that the culture system employed was efficient enough to recover high value nutrients (like EPA and DHA docosahexaenoic acid) from the fish feeds into the tissues of the polychaetes that would otherwise be vanished from the culture system. This may provide useful information to be use as a supplement or decrease the consumption of fishmeal in the aquaculture industry.

Reproduction in polychaetes is controlled by both exogenous as well as endogenous factor (Scaps 2002). Temperature and the lunar cycle are considered to be the two most important exogenous factors controlling spawning. In *H. diversicolor* spawning generally takes place in early spring and the attainment of a threshold temperature or a sudden change in temperature (Orton 1920; Thorson 1926) act as the initiation cue for spawning which typically occurs between 5°C - 11°C after a period of low temperature (Dales 1950), coinciding with either a new or full moon (Bartels-Hardege and Zeeck, 1990). During reproduction the female remain inside the burrow, the male releases sperms in front of the burrow and fertilization takes place inside the burrow where the eggs were already spawned. Development of the larvae takes place inside the burrows where they feed upon the maternal remains until they are able to exit the burrows and adapt to adult life (Marty and Retière, 1999). Moreover, as for the breeding ground, sand is the best sediment type for *H. diversicolor* as it decreases the risk of hypoxia that is associated with finer sediments, especially those rich in organic matter. In addition, sand allows easier observation and collection of cultured worms.

Bivalve filter feeders are known to consume phytoplankton such as diatoms and dinoflagellates (Shumway et al. 1987). They also consume smaller plankton like bacteria, viruses and micro-zooplankton as well as dissolved particulate organic material (which may encompass variety of microorganisms), manifesting its potential to concentrate/remove pathogens (Gosling 2003). Studies based on integration of biofilters such as mussels and oysters in fish farms has been performed in a number of countries, including Australia, USA, Canada, France, Chile and Spain In fact, several authors have found that shellfish (oysters and mussels) when co-cultivated or grown with salmon, significantly enhanced shellfish rates (MacDonald et al. 2011; Hand_a et al. 2012; Lander et al. 2013). The efficiency of the oyster *Saccostrea commercialis* in reducing the total suspended solids, and total N and P was demonstrated with affirmative results (MacDonald et al. 2011). Besides the use of bivalves as bioremediators they can also be a potential alternative to employ as fishmeal and fish oil for the aquaculture industry. From nutritional viewpoint, bivalves are known to have a high protein content and are a rich source of essential omega-3 fatty acids including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Adarme-Vega et al. 2012; Tacon and Metian, 2013). However, fluctuations in nutritional profile of bivalves according to season and geographical location were seen (Baek et al. 2014). Reproduction in bivalves is governed by both exogenous (like temperature, salinity) and endogenous factors (condition of the animal which in turn is affected by feeding).

Sea cucumbers are benthic scavengers and they have the capacity to directly ingest and assimilate waste particulate particles as well as bring about horizontal redistribution of nutrients through sediment bioturbation caused by their movement as they feed across the benthos, thereby, minimizing benthic impacts of aquaculture on one hand and increasing the benthic primary productivity (Hannah et al. 2013; Hauksson 1979; Orr, 2012; Slater and Carton, 2009; Uthicke, 1999, 2001; Yuan et al., 2015). However, the suspended filter-feeding capacity in sea cucumber is generally limited to small particles suspended in the water column, thereby making them unable to process larger particulate size and also by the spatial and temporal variations in concentration of the particles brought about by their movement through the water column (Filgueira et al. 2017; Lander et al. 2013; MacDonald et al. 2011; Reid et al. 2009; 2010). Most of the sea cucumber species are dioecious, but they do exhibit a number of different reproductive strategies with some even undergoing gender reversal (Sewall 1994). It has been suggested that physical and mechanical treatments are effective methods for induced spawning in many temperate and tropical sea cucumber species (Battaglene et al., 2002; Costelloe, 1985; Ramofafia et al. 2003), however, the results may vary and responses species-specific (Abdel Razek et al. 2012; Agudo, 2006; Domínguez-Godino et al., 2015; Hu et al. 2010; Kumara et al. 2013; Zacarías-Soto et al. 2013).

Fish

Fishmeal (FM) and fishoil (FO) represent two of the main ingredients of marine fish feed owing to their optimal nutritional characteristics, in particular: high digestibility; high content of both proteins (62-72 % as-fed) and lipids (7.6-10.2 % as-fed in FM); complete aminoacidic spectrum and optimal fatty acids composition (NRC, 2011). However, these two ingredients represent also an important bottleneck to the development of sustainable aquaculture systems (Naylor et al., 2009, 2000). In the SIMTAP system, DFFOs play a pivotal role in keeping the quality of water and as partial substitute of FM and FO in fish feed. Any novel feed ingredient must be carefully evaluated regarding the gross biochemical composition, aminoacidic, and fatty acid profiles before its inclusion in fish diet, in order to allow optimal fish grown and development. For this evaluation, we considered the nutritional requirements of marine fish species selected in the Task 1.2: *Sparus aurata*, *Dicentrarchus labrax*, *Mugil cephalus*, *Diplodus puntazzo*, *Seriola dumerili*, *Umbrina cirrosa*, *Argyrosomus regius* and *Psetta maxima* (see attachments). Then, a detailed review of the gross biochemical composition, aminoacidic and fatty acid profiles of some DFFO was carried out. The choice of DFFO included in this study is based on the results of the literature survey on polychaetes and shellfish (Deliverable D1.07) and some experiments conducted at the University of Pisa (Task 2.1): *Hediste diversicolor, Mytilus galloprovincialis, Ruditapes philippinairum*, and some Holothurians. Feed composition in terms of ingredients and biochemical composition was also reviewed.

We focused on the gross biochemical composition (**Tab. 5**), the amino acidic profile (**Tab. 6**) as well as the fatty acid profile (**Tab. 7**). These results, obtained by literature, are referred to DFFO cultured by multi-trophic aquaculture, indeed this condition is close to what we can have in SIMTAP. When these data were not available, the composition of wild DFFO was used. Some missing data (e.g., carbohydrates and energy) were also calculated by applying some formulas (Henken et al., 1986). In order to determine the theoretical substitution rate of FM and FO in marine fish diets, an electronic sheet (Excel, Microsoft) for feed formulation was built (see attachments). This tool can compare some information about nutritional requirements of fish, notably Gilthead Sea Bream and European Sea Bass, since they represent the most important species for Mediterranean area, and the biochemical composition of feed ingredients. Using this software could be possible to formulate a proper diet for the selected species at different growth stages. The database for this software is the open access International Aquaculture Feed Formulation Database (IAFFD, https://www.iaffd.com/).

Tab. 5. Proximate composition of detritivores and filter-feeder organisms.

Species	Value	Moisture (g kg^{-1} WW)	Protein	Lipid	Carbohydrate	Ash	Unit	Reference
N. diversicolor	Min		540.0	110.0	170.0	110.0	g kg^{-1} DW	(Wang et al., 2019b)
	Avg		562.0	131.7	186.0	122.0		
	Max		600.0	160.0	200.0	140.0		
	Min	791.6	420.9	118.3	250.4	65.4	g kg^{-1} DW	(Wang et al., 2019a)
	Avg	797.9	448.7	143.0	280.3	134.3		
	Max	805.2	469.5	162.1	373.0	166.6		
	Min		86.1	8.5			g kg^{-1} WW	(Santos et al., 2016)
	Avg		87.1	17.6				
	Max		88.7	22.5				
	Min		418.2	115.5			g kg^{-1} DW	(Pajand et al., 2017)
	Avg		488.7	162.5				
	Max		554.6	203.5				
M. galloprovincialis	Min	780.0	549.3	60.3			g kg^{-1} DW	(Çelik et al., 2012)
	Avg	820.0	650.8	120.3				
	Max	860.0	752.3	180.3				
	Min		313.0	19.0	18.0		g kg^{-1} DW	(Bressan and Marin, 1985)
	Avg		419.8	68.4	157.7			
	Max		523.0	138.0	316.0			
	Min	833.3	75.3	7.4		2.4	g kg-1 WW	(Azpeitia et al., 2016)
	Avg	859.3	99.6	11.0		3.0		
	Max	890.0	110.8	15.1		4.2		
	Min	790.0	65.0	14.0		2.2	g kg^{-1} WW	(Fuentes et al., 2009)
	Avg	814.4	88.3	17.0		3.0		
	Max	838.1	100.0	20.0		3.4		

Species	Value	Moisture (g kg^{-1} WW)	Protein	Lipid	Carbohydrate	Ash	Unit	Reference
		841.0	65.8	21.5		2.6	g kg^{-1} WW	(Biandolino et al., 2019)
H. tubulosa	Min	850.4	423.5	9.7	151.9	38.2	g kg^{-1} DW	(Culha et al., 2017)
	Avg	855.6	442.7	10.2	157.1	39.0		
	Max	860.9	456.0	10.7	165.3	40.1		
H. tubulosa		838.1	445.8	7.1		46.4	g kg^{-1} WW	(Sicuro et al., 2012)
H. polii		779.7	369.9	5.5		48.2	g kg^{-1} WW	
H. polii		812.4	86.6	1.5		7.9	g kg^{-1} WW	(Aydin et al., 2011)
H. tubulosa		843.0	88.2	1.8		5.1	g kg^{-1} WW	
H. mammata		852.4	78.8	0.9		5.1	g kg^{-1} WW	
H. tubulosa		867.4	81.8	1.6			g kg^{-1} WW	(Çakli et al., 2004)
R. philippinarum	Min	779.7	77.0	8.0		2.2	g kg^{-1} WW	(Beninger and Lucas, 1984)
	Avg	836.8	96.1	10.9		2.6		
	Max	867.4	120.0	14.2		3.0		
		839.8	95.6	9.8	11.4		g kg^{-1} WW	(Dincer, 2006)
Legend: DW, Dry Weight; WW, Wet Weight								

Tab. 6. Essential aminoacid (EAA) profile of detritivores and filter-feeder organisms.

Species	Value	Lys	Arg	His	Ile	Leu	Val	Met	Phe	Tyr	Thr	Trp	Unit	References
M. edulis	Min	3.25		6.35	15.06	7.82	12.56	6.02	13.20		7.30		g kg^{-1} DW	(Both et al., 2012)
	Avg	5.51		8.54	18.88	21.87	16.61	7.14	15.82		7.58			
	Max	8.20		9.86	22.10	32.98	19.80	8.00	18.50		7.72			
M. galloprovincialis		38.7	32.2	15.8	21.9	35.3	24.4	9.2	31.3		26.6		g kg^{-1} DW	
H. tubulosa		11.1	41.4	8.2	8.8	15.5	12.3	6.2	4.8	6.3	27.4		g kg^{-1} WW	(Sicuro et al., 2012)
H. polii		7.7	35.8	8.8	7.2	13.7	13.7	6.2	7.2	10.3	18.7		g kg^{-1} WW	
R. philippinarum	Min	2.7	2.6	1.1	1.6	2.7	1.9	0.6	1.6	1.4	1.7		g kg^{-1} DW	(Yin et al., 2019)
	Avg	2.9	2.8	1.2	1.8	2.9	2.1	0.8	1.7	1.6	1.9			
	Max	3.1	3.2	1.2	2.0	3.3	2.3	1.0	1.8	1.8	2.1			

Legend: DW, Dry Weight; WW, Wet Weight; Lys, Lysine; Arg, Arginine; His, Histidine; Ile, Isoleucine; Leu, Leucine; Val, Valine; Met, Methionine; Phe, Phenylalanine; Tyr, Tyrosine; Thr, Threonine; Trp, Tryptophan.

Tab. 7. Essential Fatty acid (EFA) profile of detritivores and filter-feeder organisms.

Species	Value	FA (g kg^{-1} DW)	LC-PUFA	n3 LC-PUFA	EPA	DHA	ARA	Unit	References
N. diversicolor	Min	19.00	7.05	6.45	5.27	0.22	0.60	g kg^{-1} DW	(García-Alonso et al., 2008)
	Avg	32.33	7.95	6.76	5.68	1.08	1.19		
	Max	48.00	8.76	6.97	6.23	1.58	1.91		
	Min	17.80		251.00	210.00	0.00		g kg^{-1} WW	(Bischoff et al., 2009)
	Avg	24.78		312.25	275.50	36.75			
	Max	27.10		387.00	387.00	58.00			
	Min		78.20	38.20	11.70	25.90	40.00	g kg^{-1} FA	(Lillebø et al., 2012)
	Avg		108.73	63.50	13.07	50.43	45.23		
	Max		164.80	110.10	15.20	94.90	54.70		

Species	Value	FA (g kg^{-1} DW)	LC-PUFA	n3 LC-PUFA	EPA	DHA	ARA	Unit	References
	Min		110.90	85.90	18.70	67.20	12.50	g kg^{-1} FA	(Santos et al., 2016)
	Avg		120.03	99.47	25.60	73.87	20.57		
	Max		131.20	118.70	31.50	87.20	25.00		
	Min			9.70	3.30	6.40		g kg^{-1} FA	(Pajand et al., 2017)
	Avg			43.20	27.70	15.50			
	Max			69.20	45.50	23.70			
	Min		0.85	0.73	0.11	0.37	0.12	g kg-1 DW	(Marques et al., 2018)
	Avg		9.92	9.07	6.21	3.27	0.85		
	Max		27.91	26.81	16.20	10.61	1.61		
	Min	41.16	282.30	241.80	228.20	13.60	37.40	g kg^{-1} FA	(Wang et al., 2019a)
	Avg	41.37	297.65	258.70	244.55	14.15	38.95		
	Max	41.57	313.00	275.60	260.90	14.70	40.50		
M. galloprovincialis	Min		25.20	14.20	2.20	12.00	9.00	g kg^{-1} FA	(Prato et al., 2019)
	Avg		26.90	22.13	5.57	16.57	10.00		
	Max		28.60	32.60	10.90	21.70	11.00		
	Min		254.00	223.00	75.00	148.00	31.00	g kg^{-1} FA	(Pirini et al., 2007)
	Avg		381.67	340.33	110.67	229.67	41.33		
	Max		451.00	403.00	133.00	271.00	48.00		
H. tubulosa	Min		292.10	154.70	51.00	93.20	125.20	g kg^{-1} WW	(Aydin et al., 2011)
	Avg		311.60	177.50	57.53	119.97	134.10		
	Max		333.90	208.70	61.50	148.60	139.70		
H. polii	Min		241.30	106.60	46.40	54.50	128.90		
	Avg		316.87	152.13	72.47	79.67	164.73		
	Max		378.50	201.90	93.40	124.30	230.60		
H. mammata			274.80	152.90	49.90	103.00	121.90		

Species	Value	FA (g kg^{-1} DW)	LC-PUFA	n3 LC-PUFA	EPA	DHA	ARA	Unit	References
H. tubulosa				208.60	154.30	54.30		g kg^{-1} DW	(Sicuro et al., 2012)
H. polii				182.50	129.40	53.10			
R. philippinarum	Min		154.00	127.00	27.00	84.00	27.00	g kg^{-1} FA	(Bonaldo et al., 2005)
	Avg		167.75	139.00	44.00	95.00	28.75		
	Max		202.00	169.00	68.00	101.00	33.00		
	Min	1.70	94.00	67.00	8.00	51.00	14.00	g kg^{-1} FA	(Fernández-Reiriz et al., 2006)
	Avg	2.74	149.14	129.71	11.14	118.57	19.43		
	Max	4.70	176.00	162.00	16.00	154.00	27.00		
	Min		82.00	41.00	4.00	34.00	20.00	g kg^{-1} FA	(Caers et al., 1998)
	Avg		216.50	177.63	13.88	163.75	38.88		
	Max		311.00	291.00	22.00	269.00	63.00		
	Min		135.90	49.10	10.70	38.40	54.40	g kg^{-1} FA	(Yin et al., 2019)
	Avg		160.97	71.54	11.86	59.69	89.43		
	Max		184.70	100.20	13.70	87.90	106.10		

Legend: DW, Dry Weight; WW, Wet Weight; FA, Fatty acids; LC-PUFA, long chain polyunsaturated fatty acids; EPA, eicosapentaenoic acid 20:5n-3; DHA, docosahexaenoic acid 22:6 n-3, ARA, arachidonic acid 20:4 n-6.

Task 1.4 Designing, building and trying out an integrated smart monitoring and control system conceived specifically for SIMTAP systems, allowing the partners to have a remote access for data monitoring and download, and system control (M1-M6) Task leader: UNIBO/Partner involved: UNIPI.

Work performed and main results

The process of ISMaCS design included two main activities: study of the specific needs and requirements of the experimental sites and study and research on sensors and monitoring systems.

The first activity was carried out in continuous contact with the partners involved in the on-site experiments and has taken into consideration their feedback to iteratively revise and fine-tune the design of the system. The following issues have been surveyed and taken into consideration: i) the specific characteristics of the experimental sites; ii) the planned activities; iii) the planned research and expected results.

The above-listed phases allowed to define the best technology for the monitoring of the experiments, to identify differences and similarities among the various sites, thus covering a wide range of conditions allowing the system to be flexible for future applications in productive contexts, as well as to identify the metrics to be measured and finally to define the accuracy and sensitivity of the sensors.

The second activity focused on the identification of the most suitable sensor systems and on the design and definition of the most effective, efficient and cost-effective solutions, specifically designed and built for the SIMTAP project.

Since the market did not offer any commercial solution that meet the project requirements, the ISMaCS was designed, engineered and built as a system capable of measuring the main environmental outdoor and indoor data, DO, pH and conductivity, illuminance and PAR and other quantities, and to manage and remotely send the acquired data in real time (**Fig. 9-10**).

A prototype was built and, during the SIMTAP construction, installed in the UNIPI SIMTAP system for first tests and calibration (**Fig. 11**). To run the tests, specific informatic codes were developed to remotely and automatically manage and process the data. The codes were then implemented in a routine procedure installed in the UNIBO servers. Once all the SIMTAPs will be ready, besides the tests, the calibration and validation procedures, the routine will return uniform, organized and clean data, ready to be used by the partners involved in the data analysis. The codes were designed to be the base for the data analyses for the energy efficient assessments. At the same time, the management of data has been developed both in an UNIBO and a cloud server to allow all the partners involved in the project to remotely monitor the data in real time and to have a data backup for the project.

The successful completion of the tests allows the implementation of the ISMaCS in the experimental sites (nodes and gateway) as soon as permitted. The COVID restrictions, causing delays in the delivery of the materials needed to produce the ISMaCS and strongly limiting travels (in particular abroad), have caused delays in the ISMaCS development and test, also affecting time scheduling of the implementation in the pilot sites and T2.3 activities.

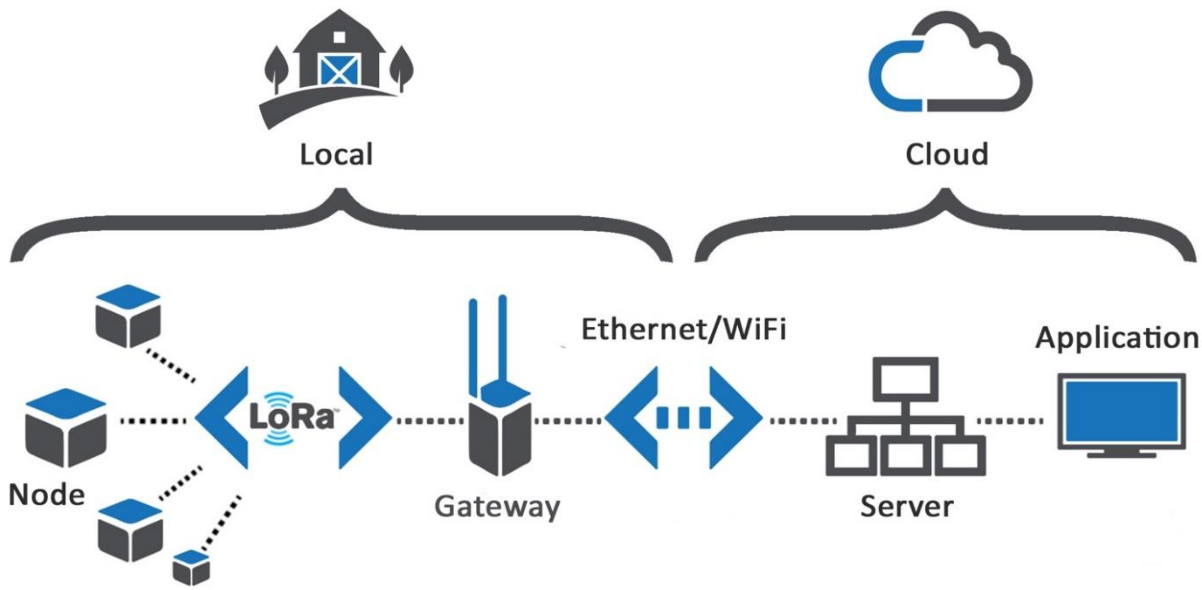


Fig. 9. Scheme of ISMaCS and communication structures

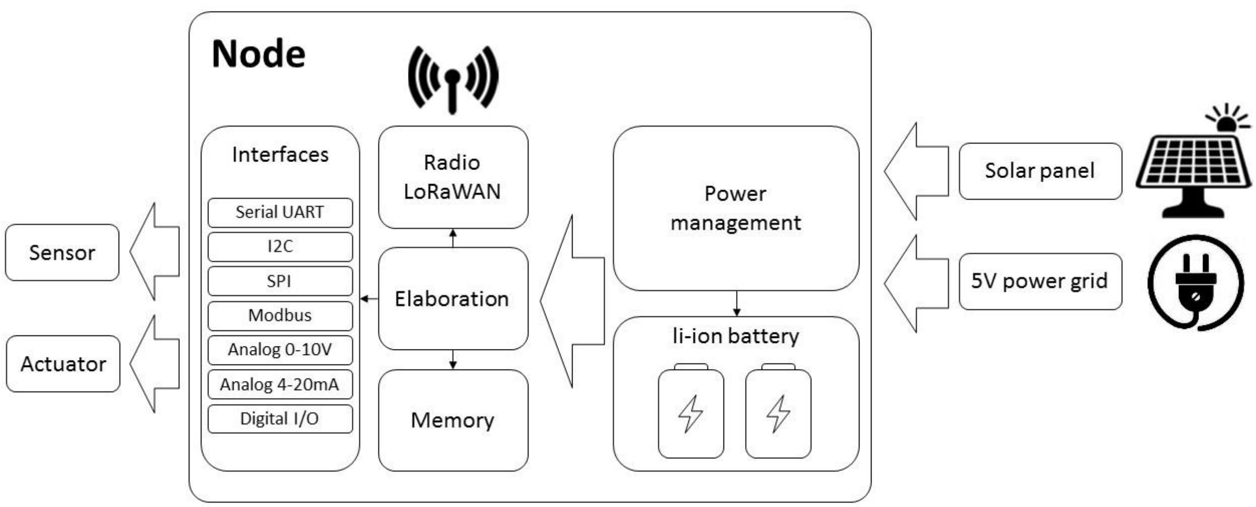


Fig. 10. Scheme of node structure with a focus on energy management, communication technology, data management and external device (sensors and actuators)

Fig. 11. Pictures of the ISMaCS prototype installed at the University of Pisa: a. Node prototype (case, PV and probe); b. node prototype (electronic board); c. Gateway (electronic board); d. Prototype during calibration test.

Deliverables

Code	Title	Delivery date MONTH	Partner	Dissemination level (PU, CO)	Type	Status (completed, delayed, in progress)	Reason for the delay
D1.1	SIMTAP design	4	UNIPI	PU	Prototype	Completed	
D1.4	SIMTAP prototype	21 (6)*	UNIPI	PU	Prototype	Completed	The prototype was completed in summer 2020 due to the lack of funds in 2019 and the Covid-19 pandemic in 2020
		21 (6)*	INRAE	PU	Prototype	Completed	
		21 (6)*	MEDFRI	PU	Prototype	Completed	
		21 (6)*	MAFA	PU	Prototype	Delayed	The system is under construction
D1.05	Report on halophyte plant species for SIMTAP production	7	UNIPI	R	Report	Completed	
D1.06	Report on algae species for SIMTAP production	3	UNIPI	R	Report	Completed	
D1.07	Report on polychaetes and shellfish in a hypothetical SIMTAP system	7	UNIPI	R	Report	Completed	
D1.09	Report on ISMaCS calibration and test: report on the ISMaCS functions and quantification of the reliability of the system	21 (7)*	UNIBO	R	Report	On time according to new schedule	Issues due to lack of funds and delays in material provision and restrictions for travelling due to COVID 19

* The expected delivery date in the proposal is shown within brackets.

Outputs

- Two manuscripts were prepared by UNIPI on the selection of the most suitable fish and plant species to be grown in the SIMTAP system using a multi-criteria decision-making method (DEXi). The manuscript on fish selection was published in an international journal while the manuscript on plant selection is in preparation.
- ISMaCS was presented by UNBO at the international virtual conference MetroAgriFor 2020 IEEE International Workshop on "Metrology for Agriculture and Forestry" (November 4-6, 2020) and at the event "European Night of Researcher" (University of Bologna, date?)
- Youtube presentation of the system (French): https://youtu.be/s3LY63kQzNM
- The IMTA rationale and SIMTAP system design and principles were presented to LML students.

1.2.2 Work Package 2

Title: Implementation and test of SIMTAP

WP Leader: MEDFRI

WP Participants: UNIPI; UNIBO; INRA UMR SAS; LML; MESDC

Start month: M7 **End month:** M30 (the end of this WP was postponed to M35)

Objectives: This WP aims to test and run SIMTAP systems installed in different environmental conditions. At the same time, SIMTAP prototypes will be run using various combination of microalgae, polychaetes and/or shellfish, finfish and halophyte plant species, in order to optimize SIMTAPs biomass production. Specifically, objectives WP2 are: i) to investigate diverse dietary inclusion levels of microalgae and deposit/filter feeders in diets for European Sea Bass, Gilthead Sea Bream and Mullet, ii) to test growth performance of microalgae, deposit/filter feeders, finfish and halophyte plant species in diverse conditions and within the SIMTAP systems, and iii) to assess and optimize the efficiency of water and energy use of the SIMTAP systems

Task 2.1: Pilot Tests. Months (from 7 to 35). Task leader: MEDFRI **Participants:** UNIPI; INRA UMR SAS; LML; MAFA

Work performed and main results

2.1.1. Studies on system start-up and bioreactor conditioning in Turkey

SIMTAP prototype start up in Turkey was made on 1 June 2020 following preliminary tests during late May. A detailed description of the prototype is given in Deliverable 1.1. The outflow of fish rearing tanks was gravitationally drained to radial flow settlers. The outlet water was discharged from the upper level of the settler into a moving bed bioreactor (MBBR). The MBBR is a rectangular tank of 1.5 m^3 (water surface volume of 1.3 m^3) stocked with biofilm carrier made of high-density polyethylene (HDPE; 500 m^2/m^3) with a 60% filling rate. Water temperature, pH, electrical conductivity, salinity, dissolved oxygen, saturation and dissolved inorganic nitrogen forms in the inlet and outlet of MBBR were monitored daily for a total of 123 days up to 2 September 2020. Because establishment of a nitrifying biofilm under saline conditions takes a relatively long period, ammonia chloride was added into recirculating water at various times to fasten bacteria growth. A commercial mixture of selected bacterial cultures (REMOVERNH3, Équo S.r.l., Prato, Italy) was inoculated on 5 July 2020 according to the manufacturer's instruction. A clear decrease of ammonia concentrations and a concomitant clear increase of nitrite concentrations following inoculation showed that ammonia oxidizing bacteria acclimatized. Then, a clear decrease of nitrite concentration representing acclimatization of nitrite oxidizing bacteria took place after a short time. Fish were stocked on 12 August 2020, but mass mortality occurred after three days due to a failure of electricity lines. Fish were restocked on 24 August 2020 with a water renewal. The concentrations of ammonia nitrogen and nitrite nitrogen were below 1.0 mg/L after acclimatization of nitrifying bacteria except for a few days occurred when mass mortality (**Fig. 11**).

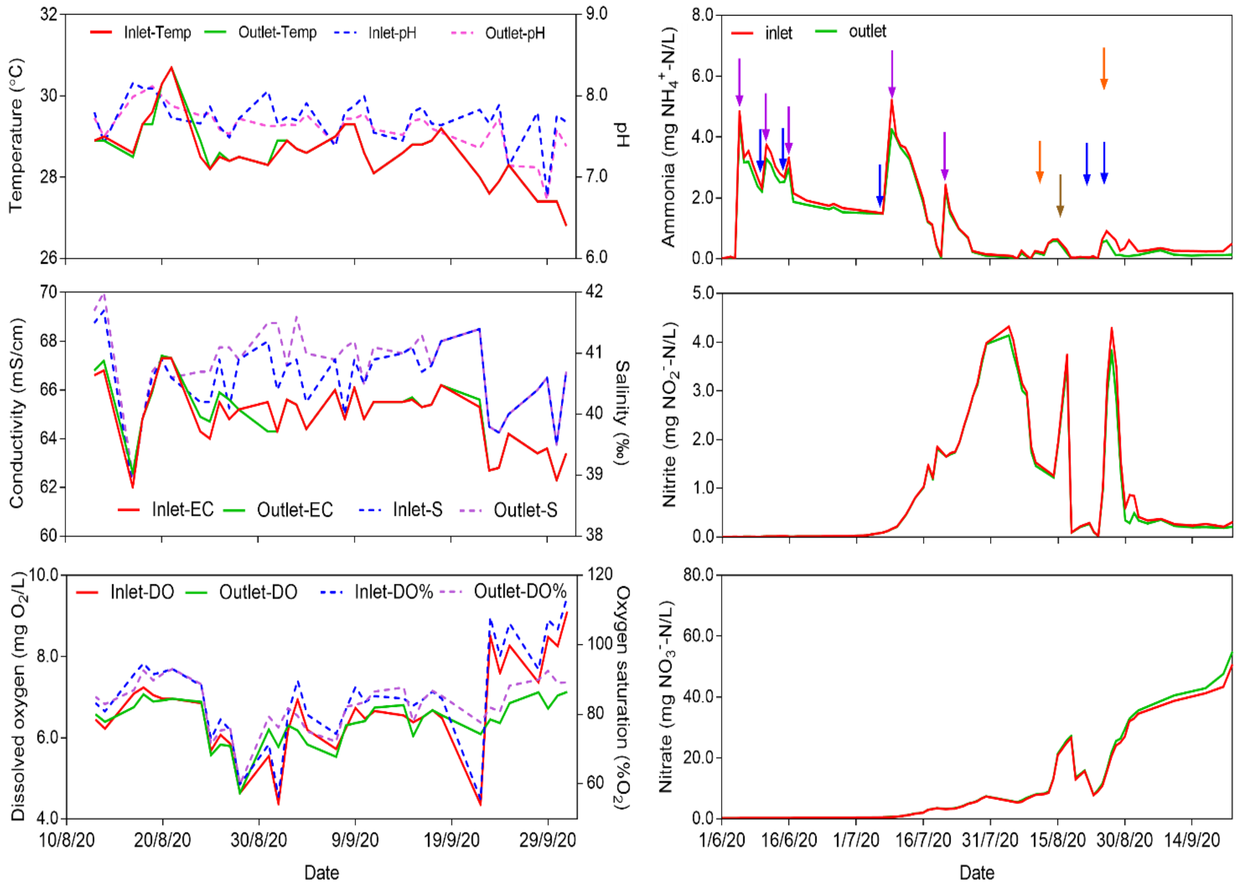


Fig. 11. Variation of water quality in bioreactor during the period of system start-up and biofilter conditioning (purple arrow: nutrient addition; blue arrow: water loss and renewal; red arrow: fish stocking; brown arrow: mass mortality).

Volumetric total ammonia conversion rate (VTR) changed between 0.10 and 33.84 g NH_4^+-N/m^3-day during the period between system start-up and biofilter conditioning period. Although a relatively high VTR values was obtained at the beginning of the period under the conditions prevailing high ammonia and low nitrite concentrations, an increasing trend was observed over about three months after the biofilter acclimatization **(Fig. 12)**

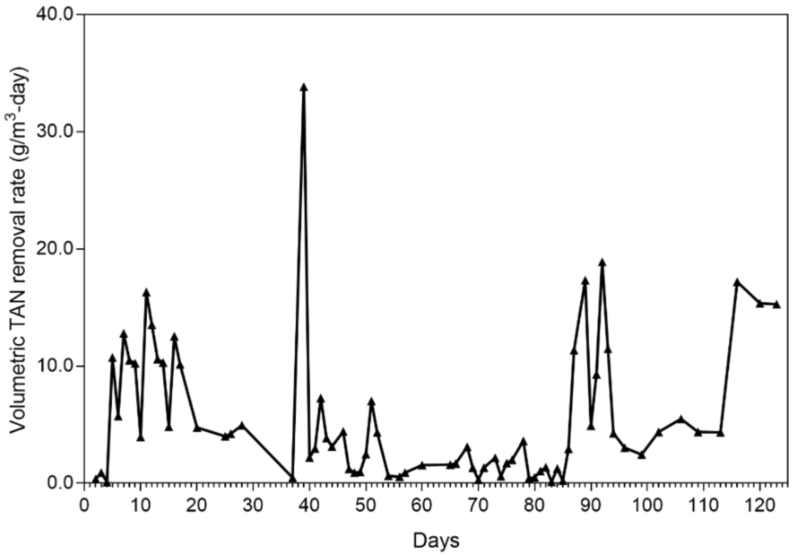
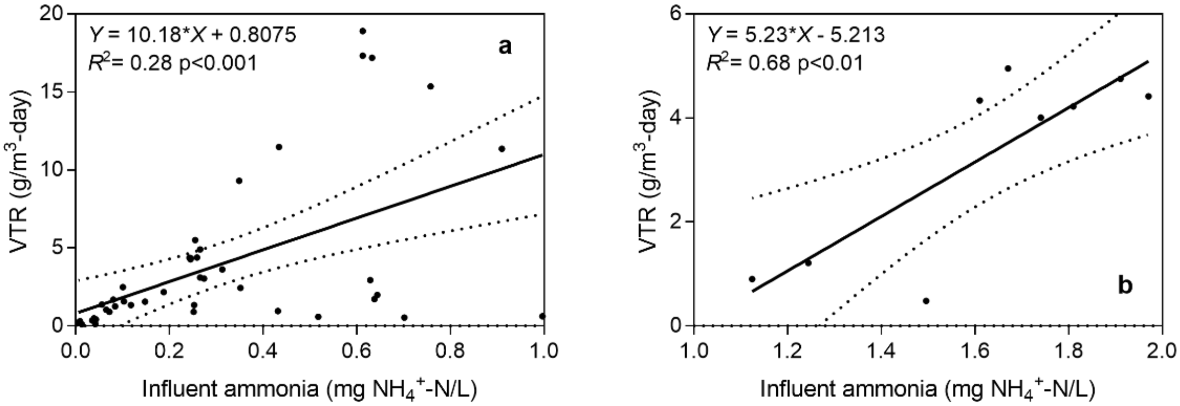


Fig. 12. Volumetric TAN removal rate in the MBBR installed

The average VTRs for low, medium and high ammonia concentrations (<1, 1-2 and 2< mg/L) in influents were 5.19±5.04, 5.04±4.93 and 5.58±6.03 g NH_4^+-N/m^3-day, respectively, with the maximum VTR of 33.8 g NH_4^+-N/m^3-day. The linear relationships between VTR and different range classes of ammonia concentrations in influent of MBBR were given in **Fig. 13.** The linear relationship between VTR and influent ammonia for low and high concentrations was relatively low (both $R^2 = 0.28$) while a strong linear relationship was determined for medium concentrations ($R^2 = 0.68$). A statistically significant difference of VTR depending on ammonia concentration was also observed (K-W test; $x^2(3) = 17.36$; $p < 0.001$). Significantly lower VTR values were obtained at the concentrations less than 1.0 mg NH_4^+-N/L compared to medium (1.0 < mg NH_4^+-N/L < 2.0) and high level (>2.0 mg NH_4^+-N/L) of concentrations (DMC test, p<0.001).

The results, in the present study, indicated a clear decrease in ammonia concentrations and a subsequent clear increase in nitrite concentrations following inoculation ammonia oxidizing bacteria over a period of one month under high temperature and salinity conditions. The complete acclimatization of nitrifying bacteria nearly took three months in the SIMTAP system built in the eastern Mediterranean conditions.



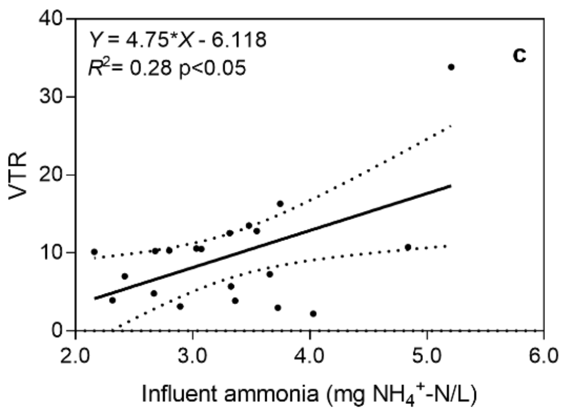


Fig. 13. The effect of influent ammonia concentration on volumetric TAN removal rate in the MBBR: a) <1.0 mg NH_4^+-N/L, b) 1.0 < mg NH_4^+-N/L < 2.0, and c) >2.0 mg NH_4^+-N/L.

Although temperature was measured between 26.8 and 30.7 °C which is also optimal for bacterial growth, the inhibiting effect of high salinity in the system on nitrification kinetics may be a reason for relatively lower removal rates in the MBBR installed compared to the literature values. On the other hand, VTR is highly dependent on ammonia and DO concentrations, and is limited by low substrate concentrations (ammonia) while by oxygen at high ammonia concentrations.

Therefore, as shown by linear relationships between VTR and ammonia concentrations, it was not surprising to observe a relatively low nitrification rates at relatively low ammonia concentrations during a short period of system start-up with immature biofilm.

2.1.2. Studies on the feasibility of growing gilthead seabream (*Sparus aurata*) juveniles using vegetable feed, supplemented with fresh mussel in France:

INRAE-LML tested the feasibility of growing gilthead seabream (*Sparus aurata*) juveniles using vegetable feed, (formulated without fishmeal and fish oil, and only composed of local vegetal raw materials) supplemented with fresh mussel (to balance the needs in micronutrient and fatty acids).

Three treatments were conducted. In the first treatment, “control” (C), the formulated feed delivered was a commercial feed, specific to marine fish at this stage of development (including marine ingredients). In the second treatment, “vegetal” (V), an experimental formulated feed was delivered and composed only of raw materials from plants, having the same chemical composition than the Control one in terms of total protein, total lipids and total digestible energy. In the last treatment, “mussel” (V+M), feed delivered was the same formulated feed than in the V treatment, supplemented with fresh flesh of mussel (shells were removed), on the basis of 1/6 of the energy supply of the feed intake. The quantity of mussels delivered was standardized according to the crude energy in mussel and in the feed from V, according to this formula:

$$quantity\ of\ mussel\ standardized\ (g) = \frac{Quantity\ of\ mussel\ delivered\ (g)\ \times crude\ energy\ in\ mussel\ (kj/g)}{crude\ energy\ in\ feed\ from\ V\ (kj/g)}$$

The fish were reared in a recirculating aquaculture system composed of three similar rearing tanks, corresponding each to one treatment (**Fig. 14**). At the beginning of the experiment, 342 fish were stocked at a mean weight of 6.8 g, in each treatment. The fish were reared during 46 days.

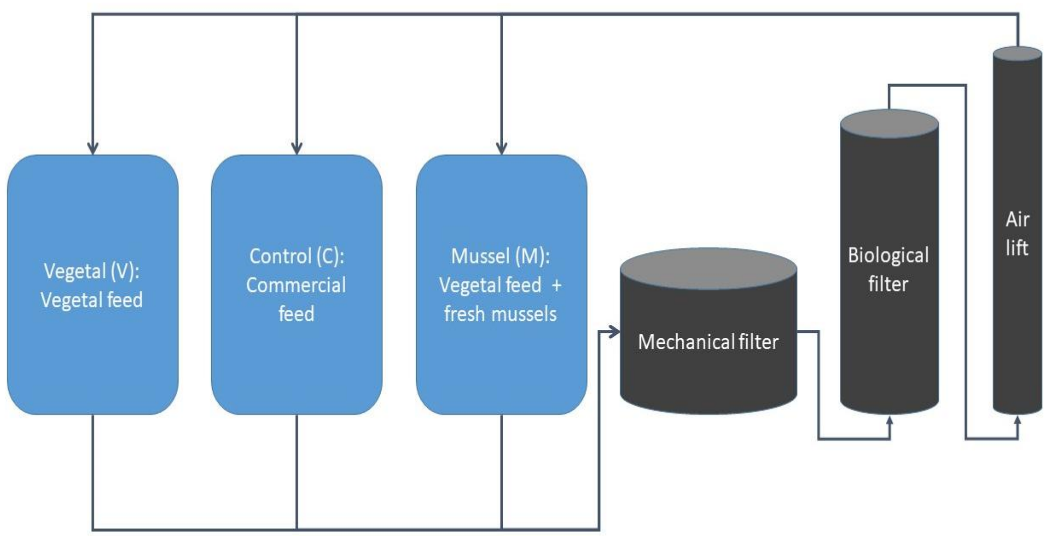


Fig. 14. Representation of the experimental design.

At harvesting, the survival rate ranged from 98% (V) to 99.7% (C). The growth was different among the treatments (**Fig. 15**). Calculated on a representative sample, the mean weights of the fish from C (23.1 ± 4.3 g, n=114) and V+M (23.1 ± 5.2 g, n=104) were significantly different ($p<0.01$) than that from V (15.5 ± 3.6 g, n=120). The harvested young gilthead seabreams were given in **Fig. 16**.

The feed efficiency for each treatment was also determined in using the feed conversion ratio (FCR) = quantity of feed delivered (g) / fish biomass gain (g). For each treatment, the quantity of feed delivered was standardized for 94% dry matter. Therefore, FCR obtained for C, V+M and V were 1.00, 0.99 and 1.84, respectively.

The body composition in fatty acids was conducted and showed a lack of EPA and DHA in the V treatment. In the V+M a slight increase in these fatty acids was observed but their level is below than the concentration in C treatment.

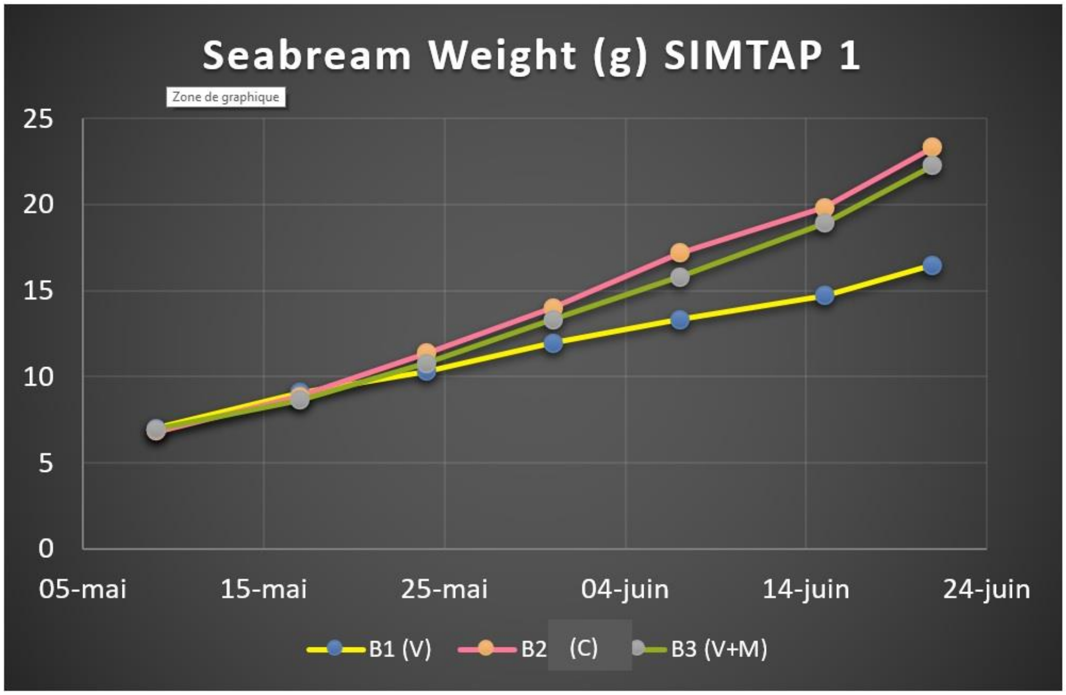


Fig. 15. Growth of seabream receiving commercial diets (C), vegetable diet (V) and vegetable diet supplemented with mussel (V+M)

Fig. 16. Young gilthead seabreams at harvesting time.

In conclusion, this experiment shows the possibility of growing seabream in using feed, formulated only with local vegetal raw materials, and supplemented with fresh mussels, while keeping performances of growth and feed efficiency, similar to that of fish fed with usual formulated feed (with fishmeal and fish oil).

2.1.3. Studies on microalgae culture in SIMTAP system by UNIPI in Italy

Microalgae are unicellular organisms that can be found both in marine and freshwater environments. In the SIMTAP system, the role of microalgae is twofold: i) they constitute feed rich in fatty acids, especially in omega-3, for the diet of DFFO, which in turn are used as feed for fish; ii) they perform wastewater phytoremediation (in particular as regards nitrogen and phosphorus) of the recirculating water in the aquaponic system or external wastewater used to reintegrate the water losses due to the evapotranspiration of the SIMTAP system.

In 2020, three experiments were performed on a laboratory scale and in sterile conditions using glass flasks placed in a growth chamber at 24/22 °C with 16/08 h day-night cycle and a photosynthetic photon flux density (PPFD) of 120 μmol/m^2.s^{-1} from cool-white light lamps. Five marine algae strains of the first experiment were cultured: *Nannochloropsis* sp., *Dunaliella* sp, *Rhodomona*s sp, *Isochrysis* sp, *Tetraselmis* sp. *Nannochloropsis* sp. strain was provided by the Maricoltura di Rosignano Solvay (Livorno) company, while the other strains were donated by the Interuniversity Center of Marine Biology (CIBM) of Livorno.

All marine strains had been grown in a specific growth medium (F medium) containing 65% of natural seawater (collected on the coast nearby Pisa) in distilled water (Guillard and Ryther, 1962). Therefore, in the first experiment each strain was cultured in an F medium that was prepared adding 35 g/L of the synthetic sea salt Instant Ocean™ (IO) to distilled water. A second experiment was conducted using F medium containing natural sea water or two different artificial sea waters, which were prepared using IO or another synthetic sea salt (Red Sea Salt™). In both experiments, algae growth was monitored by determining the optical density (OD, i.e. the absorbance at 530) and the content of dry biomass, chlorophylls and carotenoids in the growth medium.

The two experiments revealed, unexpectedly, the toxicity of IO for all the algae strains tested, although *Dunaliella* sp. was slightly more tolerant to IO than the other strains. The reason of the toxicity of IO is not clear and a specific study will be carried out in 2021, also to evaluate the need to replace IO with RSS in the laboratory cultures and in the photobioreactors of the SIMTAP system.

The third experiment with freshwater microalgae was carried out both in the laboratory (in the same conditions adopted for the previous experiment) and in the greenhouse photobioreactors of the SIMTAP system (**Fig. 17**). The freshwater algae were two strains of *Chlorella* sp. (SEC_LI_ChL_1) and C. *sorokiniana*, which belong to the algae collection of the Department of Agriculture, Food and Environment (UNIPI), and *Chlorogonium* sp., which was kindly provided by the Department of Biology. SEC_LI_ChL_1 strain was isolated a few years ago from a pond in which the leachate of a municipal landfill flowed before the remediation treatment. *C. sorokiniana* strain was collected in 2019 at the mouth of the Barra Channel, which flows into Lake Massaciuccoli (Pisa). All these strains were cultivated in a modified TAP medium at pH=7.54 (Andersen, 2005).

Fig. 17. Algae cultures in the laboratory and in the SIMTAP photobioreactors in Pisa (Italy).

The experiment in photobioreactors was conducted in the period September-December 2020. The growth medium used in the photobioreactors was prepared with commercial water-soluble fertilizers dissolved in drinking water. The SEC_LI_ChL_1 and C. *sorokiniana* strains were grown in photobioreactors to use their biomass as an ingredient in the diet of *Hediste diversicolor*, a worm species bred in the SIMTAP system. These worms can feed on both seawater and freshwater algae.

The trend of OD and the content of chlorophylls and dry biomass in the laboratory cultures are shown in **Fig. 18**. The growth of the SEC_LI_ChL_1 strain was reduced in the photobioreactors as compared to the laboratory culture. In fact, the dry biomass content of the culture medium averaged 0.17± 0.13 g L^{-1} in greenhouse photobioreactors and 3.05 ± 0.01 g/L in the laboratory. This difference was probably due to the different climatic conditions, in particular due to the lower PPFD in the greenhouse with respect to the laboratory. In contrast, no significant differences were found between the two types of cultures in terms of N and C content, which averaged 9.4 and 49.3%, respectively.

In 2020, the strain of *Chlorella* SEC_LI_ChL_1 was also characterized. The molecular characterization, based on two marker regions of DNA, was combined with observations under both optical and electron microscope (TEM) and with the determinations of some metabolic traits of algae cultures conducted under conditions of full (PPFD = 120 μmol/m^2.s) or reduced (PPFD = 60 μmol/m^2.s) autotrophy, heterotrophy and mixotrophy. The phylogenetic analysis revealed different placements within the *Chlorella-Micractinium* clade. In addition to some morphological-ultrastructural and metabolic features shared with other microalgae of the genus *Chlorella*, two peculiar characteristics were observed: the association with intracellular bacteria, never described before in these microalgae, and the presence of bicellular aggregates (also called 'doublets') not caused by cell division. This research is reported in a manuscript that was recently submitted (in revised form) to the international (peer-reviewed) journal Algal Research.

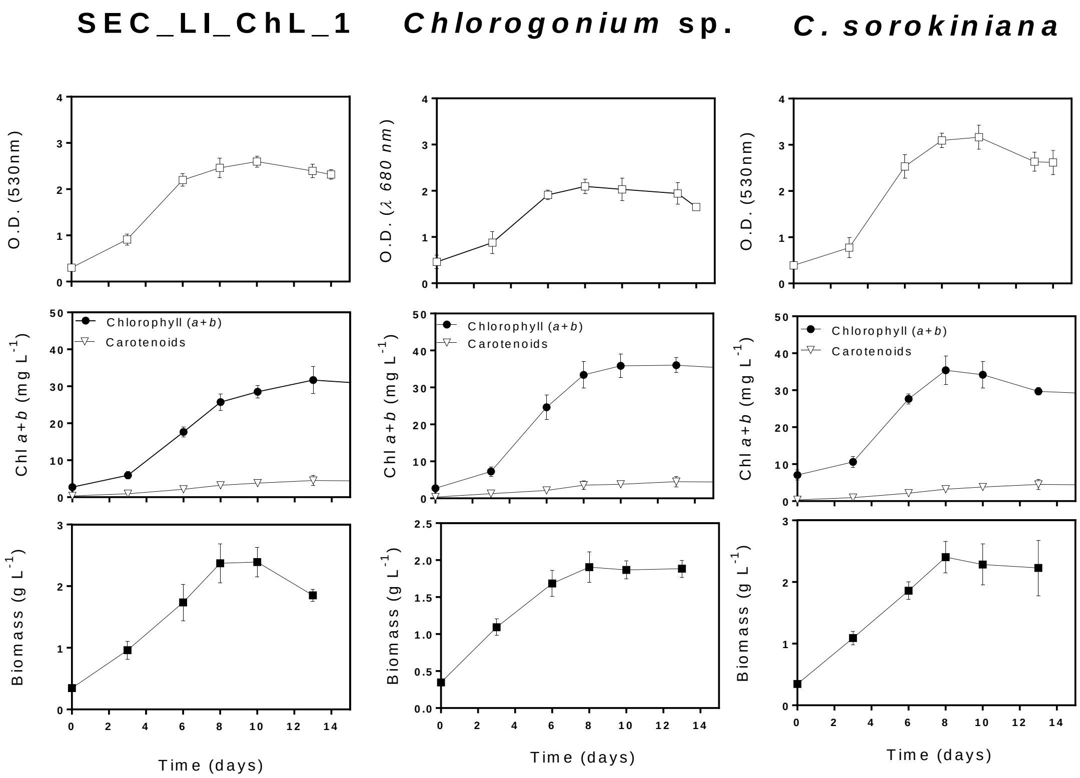


Fig. 18. Trend of optical density (OD at 530 nm) and the content of chlorophylls and dry biomass in laboratory cultures of three freshwater algae strains conducted for 13 days in sterile conditions and controlled temperature and light conditions. Mean values of three replicated (± se)

2.1.4. Studies on crop production in SIMTAP system by UNIPI in Italy

Three experiments were conducted between October 2019 and August 2020 with different plant species, which were propagated by seed and grown in a closed-loop hydroponic system with a stagnant nutrient solution (floating system). To simulate the use of sea water, different amounts of an IO were added to the control nutrient solution (total concentration of nutritive salts was about 2.6 g L-1). The IO concentrations tested in these experiments were as follows: 0 (IO-0), 10 (IO-10), 20 (IO-20) and 30 (IO-30) g/L. The species studied were: *Portulaca oleracea* L. (purslane); *Beta vulgaris* var. *cicla* (Swiss chard); *Beta vulgaris* subsp. *maritima* (sea beet); *Salicornia europaea* L. (glasswort). The first three species are glycophytes (like the vast majority of crop plants) but with a high tolerance to salinity, while glasswort is an obligate halophyte (or eualophyte).

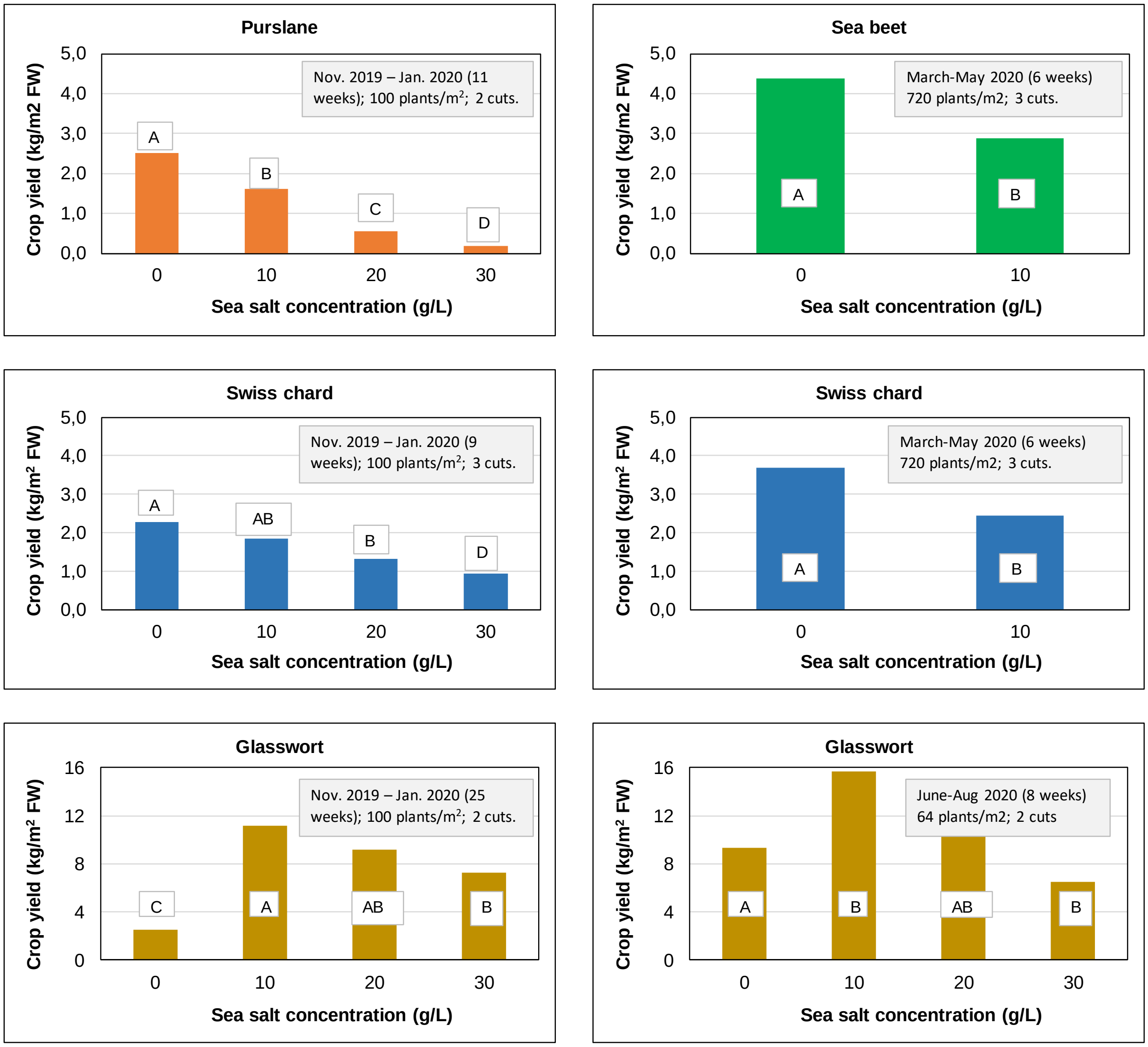


Fig. 19. Crop yield (fresh leaves or shoots) in four plant species grown in closed-loop hydroponic system under greenhouse with different concentration of synthetic sea salt Instant Ocean™ in the nutrient solution. Some information on the experiments is reported inside each graph. Mean values (n=3) keyed by the same letter are not significantly different according to Tukey test ($p<0.05$).

In experiment 1, which was conducted in autumn-winter season, crop yield decreased with increasing salinity in purslane and Swiss chard (**Fig. 19**). However, while purslane did not tolerate salinities above 10 g/L, important yield was found in Swiss chard at the highest salinities tested (**Fig. 19**). In experiment 2, Swiss chard and sea beet were cultivated using nutrient solution containing 0 or 10 g/L IO with similar results to those found in experiment 1 (**Fig. 19**).

In experiment 1, glasswort plants grew more in the grown in IC-enriched nutrient solutions than in the control; crop yield ranged between 7.24 and 11.15 kg/m^2 in saline cultures against 2.55 kg/m^2 in the control (**Fig. 19**). All the nutrient solutions were prepared with tap water and therefore the control solution contained a very low concentration of NaCl (<0.8 mM). In contrast, in summer the nutrient solutions were prepared with groundwater containing about 5.5 mM NaCl and the control plants grew well and their yield was not significantly different from that in IC-20 and IC-30 treatments (**Fig. 20)**. As found in autumn-winter season, the highest crop yield was observed in the IC-10 treatment.

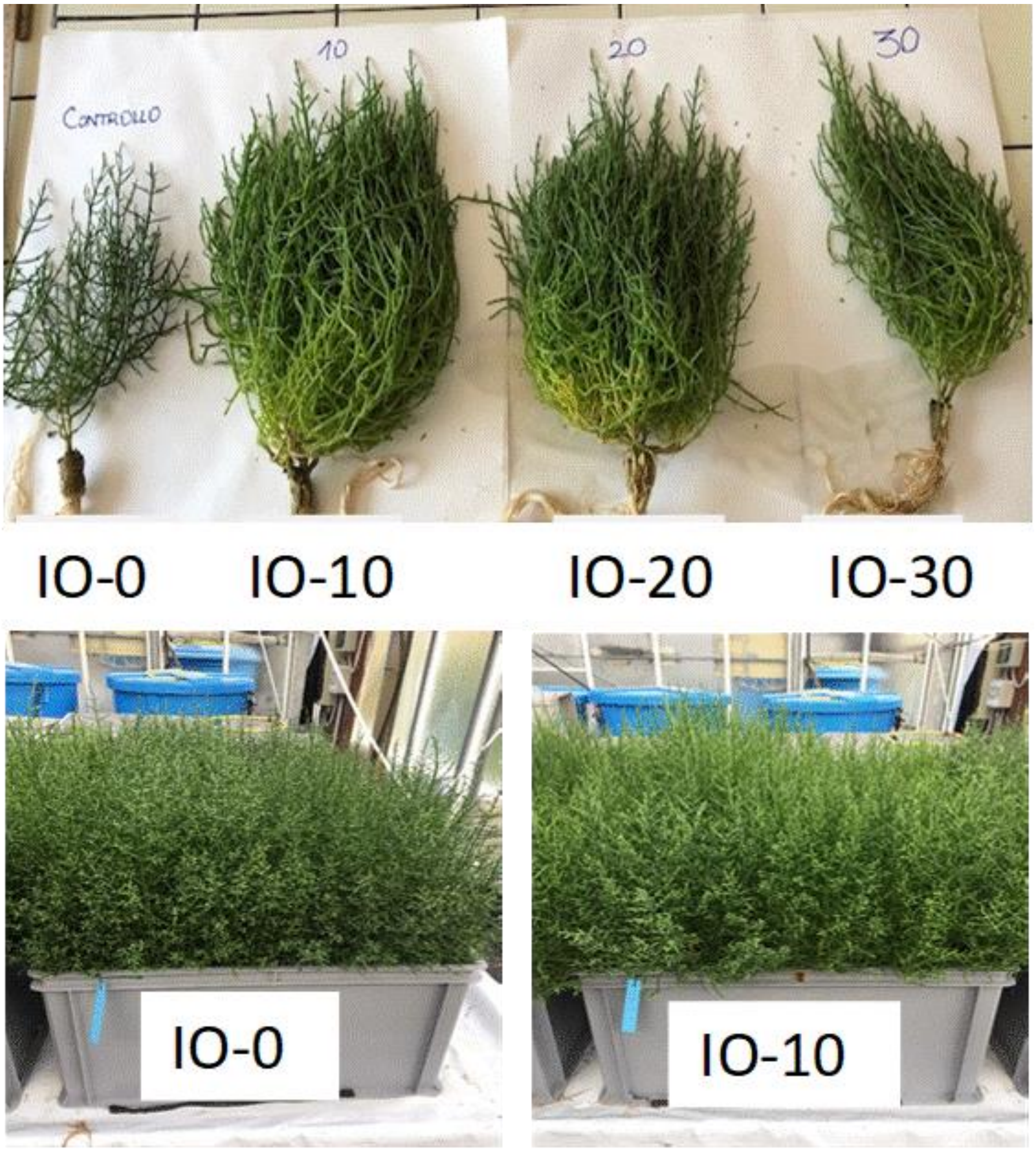


Fig. 20. Effect of the concentration of the sea salt Instant Ocean™ (IO) on the growth of *Salicornia europaea* plants grown in closed-loop hydroponic system under greenhouse in autumn-winter (top) or in summer (bottom). Legend: 0 (IO-0), 10 (IO-10), 20 (IO-20) and 30 (IO-30) g/L of IO.

Therefore, *S. europaea* is an obligate halophyte, as expected, for which however relatively low concentrations (5-6 mM) of NaCl in the nutrient solution are sufficient. The practical implications of this result are interesting. In fact, concentrations of 5-10 mM of NaCl are frequently found in irrigation water in Mediterranean regions. Therefore, *S. europaea* could be successfully cultivated in closed-loop hydroponics with available water resources with moderate salt content without any artificial salinization, which would be very expensive and would also cause a significant environmental impact caused by the recurrent discharge of exhausted saline nutrient solution. At the same time, the plant tolerates very high salinities and can be used in marine aquaponics.

Experiment 3

The hydroponic (culture of plants of agricultural interest in combination with the intensive farming of fish (aquaponics) presents some technical difficulties compared to the classic hydroponic cultivation. In fact, the presence of fish influences the composition of the nutrient solution, which often does not have the optimal ion composition for plant growth. On the contrary, in hydroponic culture the nutritional solutions are generally selected according to the cultivated species, the stage of development of the plants and the growing season as well. In marine aquaponic systems, such as SIMTAP, crops must be grown with nutrient solutions that: are very rich in salts (up to 30-35 g/L); contain low concentration of nitrogen (1-2 mM $N\text{-}NO_3$) and very high very concentrations of other nutrients (e.g. Mg and B), Na^+ and Cl^-; have abnormal molar ratios between the various nutritive elements compared to standard nutritional solutions of hydroponic crops (e.g. Mg/K ratio is much higher than usual). The main purpose of experiment 3 was therefore to analyse the growth response of Swiss chard, sea beet and glasswort cultured in hydroponics with sub-optimal nitrogen supply (low nitrate concentration in the nutrient solution) and with different levels of salinity.

In an aquaponic system, an adequate balance between the fish and plant populations is essential for normal growth of plants, which may receive insufficient amount of nitrogen, and fish, which cannot tolerate levels of ammonia and nitrite higher than 1 mg/L, and nitrate higher than 150-400 mg/L. Therefore, the nitrogen uptake by the plants was also determined.

Independent trials were carried out with three species. In each experiment, different nutrient solutions were compared with different salinities (0, 10 and 35 g/L of IO or NaCl) and nitrate concentration (1 and 10 mM, corresponding to approximately 14 and 140 mg/L of N).

Low nitrate concentration the nutrient solution reduced growth only in the two *Beta* species; in sea beet, however, this reduction was not observed when the plants were grown with the nutrient solution enriched with IO (**Tab. 7**). The growth of glasswort was significantly greater when the high salinity was obtained using IO instead of NaCl (**Tab. 7**). Total and daily uptake of nitrogen depended on plant species and salinity level, and ranged, respectively, between 10.7 and 43.2 g/m^2, and between 0.19 and 0.89 g/m^2 per day (**Tab. 7**).

Based on the results of these experiments, it can be concluded that *S. europaea* is the crop plant that best adapts to the typical conditions of marine aquaponics.

Glasswort is very interesting from a commercial point of view, as it already has an important market in some countries (France, Israel, Spain) and is increasingly present also in Italy as fresh vegetables**.** at prices as high as 9-10 €/kg. The consumption of glasswort is also promoted by gourmet cuisine and by its nutraceutical properties, although its consumption presents risks related to the high sodium content (it is also used as a substitute for table salt), oxalates and nitrates (see WP4).

Tab. 7. Crop yield (fresh weight), dry shoot biomass, nitrogen content in the dry biomass, and total and daily crop nitrogen uptake of three crop species grown in closed-loop hydroponic system with different salinity and nitrate levels in the nutrient solution.

Treatment	**[IC] o [NaCl]**	**[N-NO_3]**	**Growing period**	**Yield**	**Dry shoot biomass**	**N content of dry biomass**	**Total N uptake**	**Daily N uptake**
	g/L	mM	days	kg/m^2	kg/m^2	g/kg	g/m^2	g/m^2.day
				Beta vulgaris var. *cicla*				
IC 0-10 (control)	0	10	41	11.07 a	0.47 a	49.4 a	23,2	0,57
IC 0-1	0	1	41	7.65 b	0.42 b	40.5 b	17,0	0,41
IC 10-10	10	10	41	7.35 b	0.49 ab	50.7 a	24,8	0,61
IC 10-1	10	1	41	5.88 c	0.40 c	38.5 b	15,4	0,38
				Beta vulgaris ssp. *maritima*				
IC 0-10 (control)	0	10	41	13.11 a	0.77 a	56.1 a	43,2	1,05
IC 0-1	0	1	41	9.36 ab	0.63 b	43.8 b	27,6	0,67
IC 10-10	10	10	41	8.64 b	0.64 b	57.1 a	36,5	0,89
IC 10-1	10	1	41	7.05 c	0.55 c	38.5 c	21,2	0,52
				Salicornia europaea				
NaCl 35	35	10	56	3.30 b	0.31 a	34.4 a	10,7	0,19
IC 35-10	35	10	56	4.89 a	0.42 a	39.5 a	16,6	0,30
IC 35-1	35	1	56	4.95 a	0.42 a	35.8 a	15,0	0,27

2.1.5. Studies on polychaete breeding and nutrition by UNIPI in Italy: In the SIMTAP system, the addition of DFFO capable of extracting the organic particulate matter as well as potential pathogenic microorganisms from the recirculating water may provide an opportunity to improve the quality of the aquatic environment and reduce the risk of disease outbreaks. Moreover, the inclusion of unicellular algae as feed for the DFFO will augment the nutritional composition of these lower trophic organisms, which when fed by fish will led to the biomagnification of the polyunsaturated fatty acids (PUFAs) present in these organisms. All these will reduce the operation costs of SIMTAP system reducing the costs for water treatment and commercial feed. In this context, a multifaceted research work was conducted in 2020 on the polychaeta *H. diversicolor (*a detritivore organism) using juvenile worms obtained from laboratory cultures or juvenile and adult worms collected in the wild.

Experiment 1 (breeding)

Due to their small body mass, *H. diversicolor* worms have a predominant metabolic rate, energy demand and uptake rate (Norkko et al. 2013). Moreover, when cultured under control environment at high density, these worms can mature and reach the commercial size (10–12 cm) in around four months (Nesto et al. 2012). In fact, *H. diversicolor* is of interest owing to its use as bait in recreational fishing and as food in aquaculture. The market value of polychaetes is about 9 euro for approx. 220 g (http://www.baitsrus.com/) (Marques et al. 2017) and 20 euro/kilo for freeze dried products (http://www.topsybaits.nl). However, the main challenge lies in the breeding of this worm in a sustainable way. Successful breeding programs could assure a year-round supply of worms for the SIMTAP system. In the wild, temperature and the lunar cycle are the two most important exogenous factors controlling spawning. Many studies have shown that most of the marine animals, including some Polychaetes, respond to the relative duration of the diurnal light-dark cycle *i.e*. thereby making photoperiod to be likely the candidate for reproduction (Olive et al. 1999). Thus, we hypothesized that a transition brought about by a sudden drop in temperature and blue-light cues simulating moonlight could induce synchronous spawning in *H. diversicolor* under laboratory conditions. A drop in temperature simulates approaching winter and the sudden rise in temperature acts as a cue for spawning (Bartels-Hardege and Zeeck 1990).

Therefore, a breeding experiment was performed during August-October 2020 to determine: i) if spawning can be induced to polychaetes outside of their breeding season under laboratory conditions, ii) if lunar cycle effectively induces synchronous spawning; iii) the right time to collect larvae from the sand. The experiment consists of three different treatments *viz*. control, treatment 1 (T1) and treatment 2 (T2), each consisting of three replicates. Fifteen juveniles (0.05 ± 0.002 g fresh weight) were distributed in each of the 30-L culture tanks with 10 cm of sand (0.4 – 2.0 mm). Water flow rate was maintained at 6 L/h, and salinity and photoperiod were 17 ± 1 g/L and 16/8 light dark cycle, respectively (1.05 W/m^2, from white LED lamps). The treatment groups (T1 and T2) were exposed to a semilunar cycle simulated using blue light (0.02 W/m^2) and were given thermal shock to induce spawning in the worms. The controls, on the other hand, were cultured under uniform photoperiod (16/8 light-dark cycle) at room temperature. During the experiment, worms were fed *ad libitum* on mussels, thrice a week. Uneaten feed and faecal matter were siphoned off before each feeding.

The first mature worms (indicated by green coloration) appeared after 4 weeks. At this time, worms from treatments T1 and T2 were transferred to a chiller set at 6 ± 1°C for 7 days, thereafter they were transferred back to room temperature. After 6 weeks, all the individuals visible in the control and in weeks after the cold shock. T1 were counted and their lengths measured. Treatment T2 was similar to T1, but they were sampled two weeks later. In all the treatments, the collected larvae were divided into three groups according to their lengths (**Fig. 21**): <5 mm (L1), 5-10 mm (L2) and >10 mm (L3).

One-way ANOVA and Tukey's multiple comparison test (P<0.05) were performed using GraphPad Prism 5 to compare the larval groups obtained and unspawned worms that remained.

The percentage of spawning females was 64%, 97.7% and 95.5% in control, T1 and T2 treatments, respectively. The controls consisted of larvae of all sizes, whilst in T1 and T2 the predominant larvae size was, respectively, L1 (<5 mm) 81% and L2 (5-10 mm) 75% (**Tab. 8**). The number of adult worms that remained at the end of the experiments were counted too. The highest number of unspawned adults was found in control (**Tab. 8**).

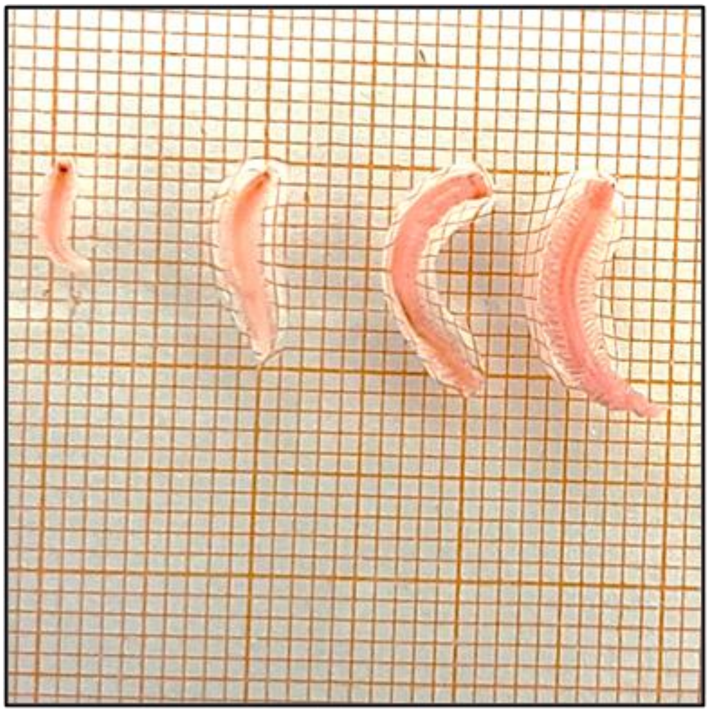


Fig. 21. Larvae with different sizes. From left: <5 mm (L1), 5-10 mm (L2) and >10 mm (L3)

Tab. 8. Larval size (L1: < 5 mm; L2: 5-10 mm; L3: >10 mm) and unspawned adults of *H. diversicolor* harvested at different time-period after thermal shock treatment

Treatment	**Treatment length (days)**	**Thermal shock**	**Moonlight**	**L1 (%)**	**L2 (%)**	**L3 (%)**	**Unspawned worms**
Control	42	No	No	17^{b}	44^{ab}	39	16^{a}
T1	42	Yes	Yes	81^{a}	19^{a}	0	1^{b}
T2	56	Yes	Yes	10^{b}	75^{b}	15	2^{b}

Different superscripts from a to b within columns indicate significant difference (p<0.05) according to Tukey test.

Therefore, in the control group spawning took place through the whole experiment as a mix of larval stages was observed found. Conversely, in T1 and T2 most of the females spawned at a similar time, which might be ascribed to the simulated moonlight. In addition, our study manifested the approximate larval size under the sand that can be expected within a definite time period. This will be useful during worm recruitment as one can estimated the size of the larvae with reference to spawning time of the adult worms in consideration. In **Tab. 8**, it can be clearly seen that sampling of worms approximately 6 weeks after the thermal shock resulted in larval size <5 mm whilst a delay in sampling time led to bigger larvae.

In conclusion, the lunar cycle did play an important role in synchronizing the spawning in *H. diversicolor* in agreement with the findings of Bartels-Hardege and Zeeck (1990). *H. diversicolor* can be induced to spawn under laboratory conditions with blue light to mimic lunar cycle and with cold shock to generate synchronous spawning in well-fed worms.

Experiment 2 (worm feeding with *Chlorella* sp.)

Chlorella-like microalgal strains are almost ubiquitous, as they are found not only in freshwater and seawater environments, but also in terrestrial and aerial niches. In addition, *Chlorella* sp. is very rich in proteins, exhibits a high growth rate, and is tolerant to changing environments conditions, as previously observed in the algae laboratory at the University of Pisa. In the SIMTAP system, the polychaetes could be fed on fish waste and microalgae. As such, an experiment was carried out to investigate growth performance, survival rate and the nutritional composition of the worms fed different diets of microalgae and mussels.

The experiment was designed with a diet consisting of mussel (Chilean mussel, *Mytilus chilensis* o *Mytilus platensis*) provided by ARBI Dario S.p.A Italy, and the strain SEC_LI_ChL_1 of *Chlorella* sp., at different inclusion levels. Fifteen juvenile worms were distributed in each of the culture tanks provided with 10 cm of sand and cultured in the same conditions as in experiment 1. The worms were fed with the microalgae *Chlorella* sp. and mussels at different inclusion levels, as follows (algae:mussel): 100:0 (T1); 75:25 (T2), 50:50 (T3); 24:75 (T4); 0:100 (T5). Each treatment consists of three replicates. The algae fed group was given 3% of the worm biomass (wet weight) each day; the remaining treatments were given the carbon equivalent of the algae feed of their respective diets. At the onset and at the end of the experiment, samples of worms, microalgae and mussels were sampled for the determination of nutritional composition (content of proteins, lipid, carbohydrates and ash, and fatty acid profile) to assess how much of the nutrients contained in the algae composition can the worms retained. At the end of the experiment, worms were sampled, and their weights recorded. The algae culture was monitored by measuring the optical density (i.e. absorbance at 530 nm) and the content of dry biomass and chlorophylls. Algae biomass was collected when the optical density, which was closely correlated to the dry biomass content, reached a stable stage (**Fig. 22**).

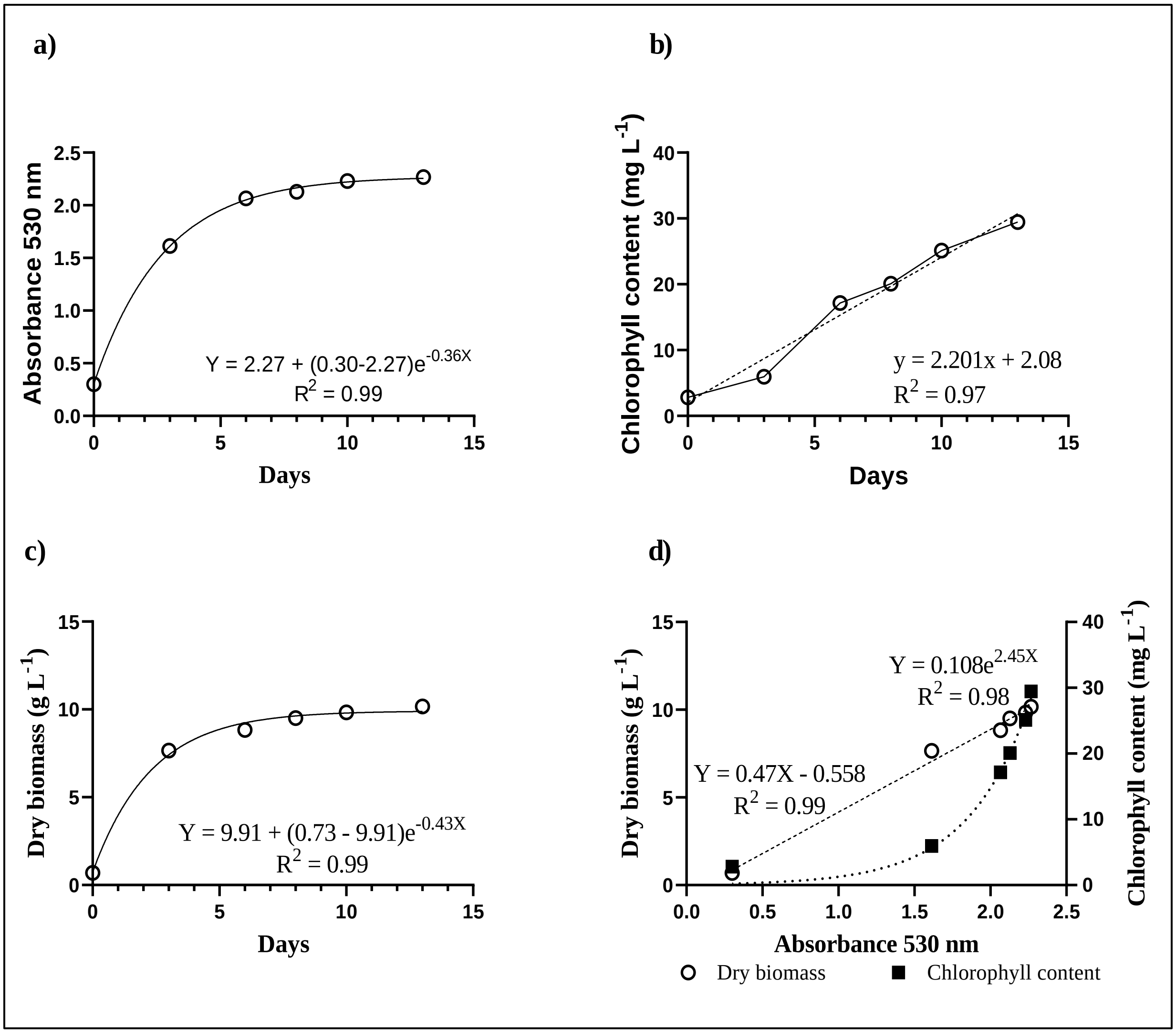


Fig. 22. Time course over 13 days of the optical density (absorbance at 530 nm, a) and the content of chlorophylls (b) and dry biomass (c) in the laboratory culture of the strain SEC_LI_ChL_1 of *Chlorella* sp.. The relationship between the optical density and the content of dry biomass or chlorophylls is shown in graph (d).

Results of the growth parameters and survival of *Hediste diversicolor* are shown in **Tab. 9**. Worms in treatment T2, T3 and T5 showed a significantly higher specific growth rate (SGR) than in the other treatments. Moreover, the final biomass in T2 and T3 was found to be significantly higher than T1. The highest survival rate was found in T2 and T3. Laboratory analyses of the nutritional composition of mussels, algae and worms are in progress.

In conclusion, the inclusion level of microalgae:mussel in the weight ratio of 75:25 and 50:50 promoted the growth of worms and increased their survival rate as compared with the other inclusion levels.

Tab. 9. Growth performance and survival percent of *Hediste diversicolor* fed with different diets based on microalgae and mussel for 5 weeks.

Treatments (algae:mussel, weight ratio)	**SGR (%/day)**	**Final Biomass (g)**	**Survival (%)**
T1 (100:0)	0.68 ± 0.16 b	0.73 ± 0.06 c	66.6 ± 1.0 b
T2 (75:25)	3.63 ± 0.55 a	7.66 ± 0.76 ab	93.3 ± 1.0 a
T3 (50:50)	4.30 ± 0.65 a	8.32 ± 1.34 a	91.1 ± 1.5 a
T4 (25:75)	1.07 ± 1.1 b	1.48 ± 0.58 bc	73.3 ± 1.0 ab
T5 (0:100)	3.20 ± 0.88 a	6.04 ± 0.84 ab	80.0 ± 1.0 ab

In the columns, means (n=3, ± s.d.) followed by the same letter are not significantly different according to Tukey test ($p<0.05$).

Work in progress

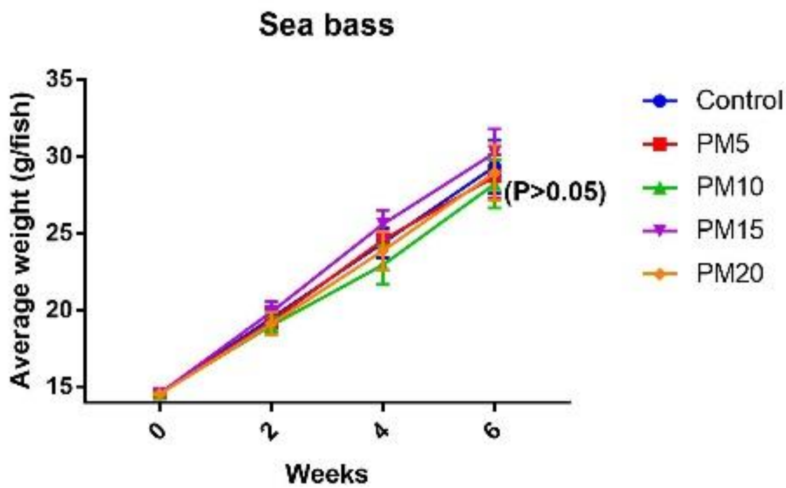
2.1.6. Studies on evaluation of nutritional value of polychaete meal for European sea bass (*Dicentrarachus labrax*) and gilthead sea bream (*Sparus aurata*) in Turkey.

Since polychaete fed with fish wastes generated in the integrated system will be used for feeding fish again, its nutrition composition and nutritional value should be studied beforehand. For this reason, two experiments were planned in European sea bass and gilthead sea bream. Feed ingredients were obtained from the local market and their compositions were determined. Polychaete material was obtained from the Aegean Sea at different times as frozen or alive. Material arrived as frozen was dried in a freeze dryer whereas those arrived alive was dried in an oven at 65°C for one day. Following drying process, the two forms of polychaete meal (PM) were combined at ratio about 1:1 to include into the experimental diets.

European sea bass: PM was incorporated at 0%, 5, 10, 15 and 20 at the expense of fish meal and fish oil. The diets were formulated to be isoproteic (48%) and isolipidic (14%). The ingredients were thoroughly mixed and then deionized water was added until dough like consistency. The mixtures were extruded using a meat chopper at 2 mm diameter. The resulting pellets were broken into small pieces, dried at 65°C and stored in airtight bags until used.

Gilthead sea bream: PM inclusion levels are the same with sea bass diets. The diets were formulated to contain 48% protein and 12% lipid. Diet processing is also the same above.

Fish material used in the experiments were procured from a private farm. They were randomly allocated to 400 L tanks and adapted to experimental conditions for 4 weeks. At the start of the experiments (05 November 2020), 10 fish with homogenous size were remained in the tanks. Initial average weights of sea bass and sea bream were 14.56±0.01 and 20.03±0.02 g respectively. Each experimental diet was tested triplicated tanks. Fish were fed until apparent satiation twice a day. Growth performance was monitored with biweekly weightings. Both experiments have reached 6th week and the growth trajectories obtained from the last weighing (19 December 2020) are given in **Fig. 23**. The growth data of fish on experimental diets were compared with the one way ANOVA. Based on this, gradual incorporation of PM in diets instead of fish meal did not significantly change fish growth (P>0.05).



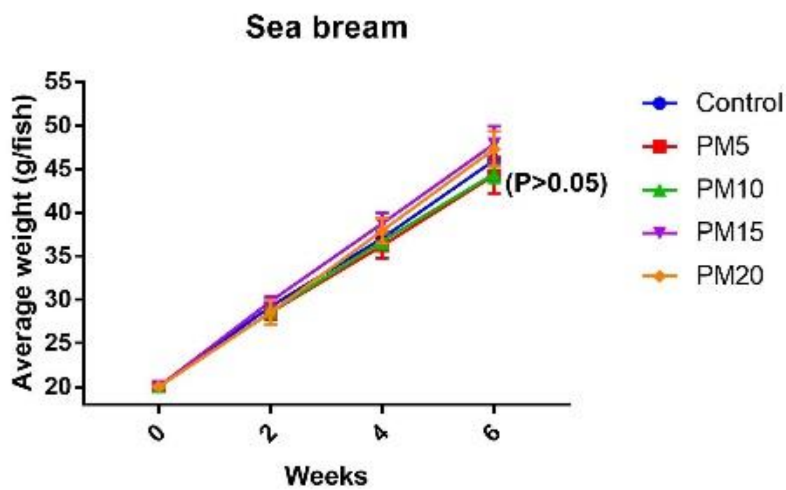


Fig. 23. Growth of European sea bass and gilthead sea bream fed varying levels of polychaete meal for 6 weeks

2.1.7. Studies on growth performance of gilthead sea bream (*Sparus aurata*) in relation to different replacement rates of commercial feed with mussels by UNIPI in Italy.

In order to reduce the use of commercial feed whose ingredients are frequently imported from all over the globe, in particular from Brazil, Argentina and USA, the use of mussels reared "*in loco*" within the SIMTAP system may represent an interesting alternative. This is particularly true for carnivorous fish species such as Gilthead Seabream (*Sparus aurata*) and European Seabass

(*Dicentrarchus labrax*). For this reason, an experiment was conducted in 2020 to investigate the fish growth performances in relation to different replacement rates of commercial feed with mussels.

To this purpose, 1243 Gilthead seabream (BW 4.95±1.12 g) were randomly allocated in six different tanks (420 L capacity each) and fed as follow (Feed/Mussels, dry weight ratio): 100/0% (control), 80/20%, 60/40%, 40/60%, 20/80% and 0/100%. The pellet diameter of the commercial feeds used (Optima, INVE Aquaculture, Belgium) were <1.2 mm until day 21, then 2 mm until the end of the trial. Frozen mussels (Chilean mussel, *Mytilus chilensis* o *Mytilus platensis*) were kindly donated by ARBI Company (Monsummano, Pistoia, Italy). Commercial feed was kindly provided by INVE, Aquaculture Rosignano, Rosignano Solvay, Livorno, Italy), which also provided fish juveniles.

Fish were fed three times per day (08:00 a.m., 10:30 a.m., and 01:00 p.m.); the feed was directly supplied while the frozen mussels were thawed, then minced with a mixer until reaching a 1-4 mm particle size and delivered to the fish tanks. Feed and mussel dry matter (DM) was 95% and 20%, respectively. Based on DM content, the daily feeding rate was 3% of the fish biomass throughout the experimental period and the total amount supplied every day was adjusted weekly according to the fish growth.

Body weight (BW) and total length were measured in all fish individuals at the beginning and the end of the experiment. Moreover, BW and total length were weekly measured on 25 fish of each treatment. Weekly and cumulated Feed Conversion Rate (FCR) was calculated as follow:

$$FCR = \ diet\,supplied\ (g\ DW)/BW\ increase\ (g, wet\ weight)$$

Growth performances of the groups were compared taking into account the differences between groups in initial body weight; thus, the data were elaborated reducing the BW of each fish by the general mean of initial BW and then adding the group BW mean. One-way ANOVA was performed, and means were compared with HSD-Tukey test (P<0.05).

Minced mussels showed a significantly higher palatability than feed, as fish competed much more for capturing mussel particles than they did for the feed particles. The trial was considered concluded after 42 days, when the initial fish BW had tripled. The fish growth performances observed for all the considered groups can be considered as standard in relation to the species, the growth stage (age) and the commercial diets regime (**Tab. 10**). Minor significant differences were observed already on day 0, when the 0/100 and 100/0 groups showed lower BW compared to other groups (**Tab. 10**). These differences tended to disappear (e.g. on day 28 and 35) while on day 42 the 0/100 group showed the lowest BW, which was significantly different from the group fed only commercial feed (**Tab.** 4). Moreover, at the end of the experiment fish BW was significantly greater in the 60/40 group than in the 100/0 group (**Tab. 10**). The 0/100 group showed the highest cumulative FCR (1.11) followed by the groups 80/20 (1.06), 20/80 (1.04), 40/60 (1.02), 100/0 (1.01) and 60/40 (0.98). All the other FCR values calculated for each period and over the while experiments are shown in **Fig. 24-25.**

Tab. 10. Body weight (g) of Gilthead seabream fish reared in the SIMTAP system and fed on diets containing different weight ratios between commercial feed and mussels.

		Treatment (Feed/Mussels, DW ratio)					
Sampling time		**0/100**	**20/80**	**40/60**	**60/40**	**80/20**	**100/0**
	mean	4.76	5.10	5.17	5.1	4.86	4.76
Day 0	s.d.	1.04	1.16	1.07	1.21	1.07	1.07
		B	A	A	A	AB	B
	m	6.07	6.60	6.75	6.65	6.78	6.49
Day 7	s.d.	1.17	1.40	1.35	1.39	1.48	1.33
		B	A	AB	A	A	AB
	m	7.38	8.09	8.34	8.19	8.71	8.23
Day 14	s.d.	1.31	1.63	1.62	1.57	1.887	1.58
		B	AB	AB	AB	A	AB
	m	8.43	9.53	9.9	9.91	9.99	9.76
Day 21	s.d.	1.74	1.43	1.62	1.93	2.14	1.72
		B	AB	A	A	A	AB
	m	11.16	11.66	10.88	12.03	11.54	11.04
Day 28	s.d.	1.65	2.30	2.52	2.21	2.25	2.34
		A	A	A	A	A	A
	m	13.28	14.28	13.78	13.73	12.56	14.06
Day 35	s.d.	2.70	2.53	2.40	1.79	2.76	2.94
		A	A	A	A	A	A
	m	13.02	15.50	15.07	15.5	14.87	14.69
Day 42	s.d.	2.60	2.89	2.97	3.32	3.53	3.49
		C	AB	AB	A	AB	B

In the rows, mean values followed by the same letter are not significantly different according to Tukey test (P<0.05).

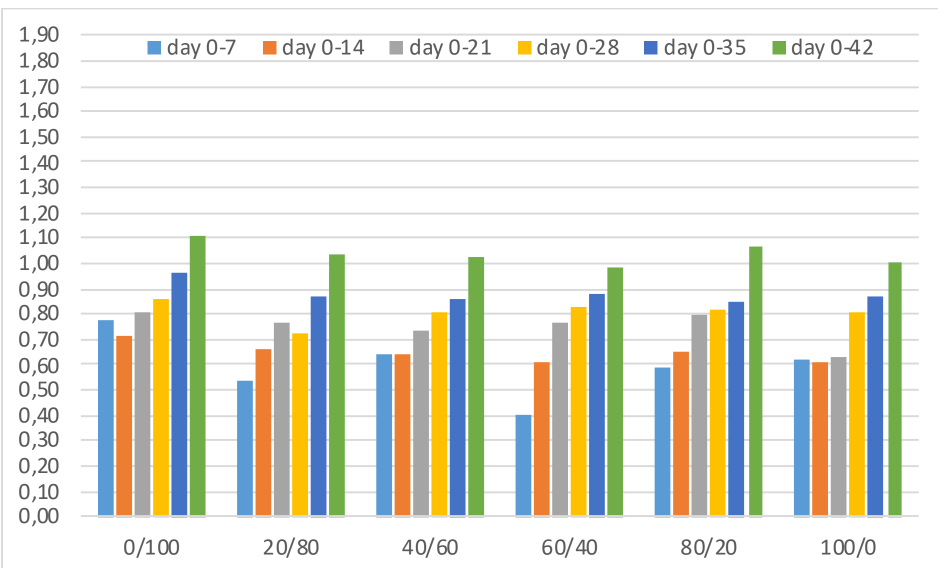


Fig. 24. Cumulative feed conversion rate (FCR) of Gilthead seabream reared in the SIMTAP system and fed on diets containing different weight ratios between commercial feed and mussels.

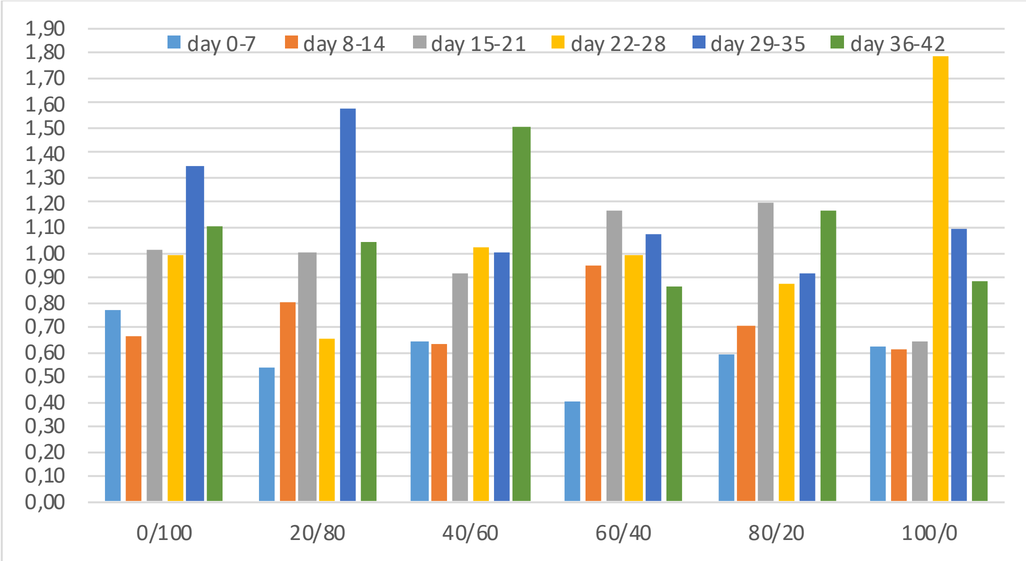


Fig. 25. Feed conversion rate (FCR) at different stage of fish growth (period FCR) of Gilthead seabream reared in the SIMTAP system and fed on diets containing different weight ratios between commercial feed and mussels.

2.1.8. Studies on microalgae culture in SIMTAP system by UNIPI in Italy: In 2021 an experiment will be conducted with glass flask cultures of *Chlorella* SEC_LI_ChL_1 strain both in the laboratory and in the greenhouse using growing medium prepared with greenhouse runoff water rich in nutritive ions and NaCl, in comparison to the standard medium (control). Subsequently, the two media will be tested in the photobioreactors. Seawater algae will be also cultured in the photobioreactors in spring-summer season. In each experiment, the production of biomass and the total content of C, N, carbohydrates, lipids and proteins will be determined. In order to evaluate microalgae *micro* as a feed ingredient for DFFO in the SIMTAP system, the content of lipids, fatty acids PUFAs and protein content will be analysed in both sea and freshwater algae.

2.1.9. Studies on crop production in SIMTAP system by UNIPI in Italy: Experiments with Swiss chard, sea beet and glasswort will be conducted in the SIMTAP system in winter and spring season to assess crop yield and quality. In this experiment, the time course of the concentration of total and inorganic nitrogen and phosphorus will be also determined.

2.1.10. Studies on polychaete culture in the SIMTAP system by UNIPI in Italy: In September 2020, polychaetes were stocked in nine tanks of the SIMTAP system at a density of 200 individual per m^2. Each thank has an approximate volume of 0.5 m^3 and a surface area of 1 m^2. The bottom of the tanks was lined with sand (up to 2 mm size).

The polychaetes were collected from the wild from Canale di Navicelli, (**Fig. 26**) by digging the soil and separating the worms manually. The salinity and the temperature of the sampling site was measured. The worms were immediately transferred into plastic tanks provided with a thin layer of artificial sand and water from the sampling site. This was done not only to avoid injury to worms during transportation but also to avoid tangling of worms. The sand provides a safe shelter for the worms. Once in the laboratory, the worms were transferred in a tank filled with 1 cm of sand and 5 cm of artificial sea water and allowed to depurate for 24 hr under optimal conditions. Afterwards, they were visually divided into three groups (*viz.* small, medium and large) and introduced into the tanks. In addition, 600 larvae obtained from the breeding laboratory (see experiment 1 and 2) were also introduced into the same tanks together with wild worms.

Fig. 26. Collection of polychaetes from the Canale dei Navicelli (Pisa).

In October, mussels *Mytilus galloprovincialis* in socks were also introduced into the polychaetes tanks. Each sock weighs around 2.8 kg. The polychaetes were also fed with faecal matter from juveniles of sea bream reared within the SIMTAP system till mid-October, when the experiment with fish was concluded. At present, the polychaetes fed on mussels. The growth of worms in the SIMTAP system will be monitored during 2021 with particular attention to the period with fish in the aquaculture tanks.

2.1.11. Studies progressing during next period in SIMTAP system in France:

More in deep analysis of the results will conduct to propose the modelling of the nutrient flows in the system. A scientific publication is expected.

A supplementary cycle of production will be carried out in 2021 to:

- Integrate production of macro-algae in the system in including the pond 2 in the loop of the system, between the ponds 4 and 7.

- Improve the system with an additional airlift set in the pond 6 to preserve shrimps from depletion of oxygen.
- Set and test the smart monitoring related to the task 1.4 of the WP1, which should improve our understanding of the system.

Task 2.2: Testing SIMTAP or its specific parts. Months (from 11 to 35). Task leader: MEDFRI Participants: UNIPI; INRA UMR SAS; LML; MAFA

Work performed and main results

2.2.1. Studies on fish growth performances at fattening stage in SIMTAP system in France: The French SIMTAP system (INRAE-LML) is represented on the **Fig. 27**. The system was tested from 9th June to 7th October 2020 in the facilities of LML. The system was developed with the aim to maximise the use of nutrients contained in the formulated feed delivered to seabream (*Sparus aurata*) in associating different species of different trophic levels. To limit environmental impacts related to the use of fishmeal and fish oil in the usual formulated feed, only local vegetal raw materials composed the formulated feed. In addition, fresh mussels, discarded from the market because of out of calibration, were supplied to balance micronutrient and fatty acids needs of fish, at a rate of 1/6 on the basis of the energy content of the formulated feed.

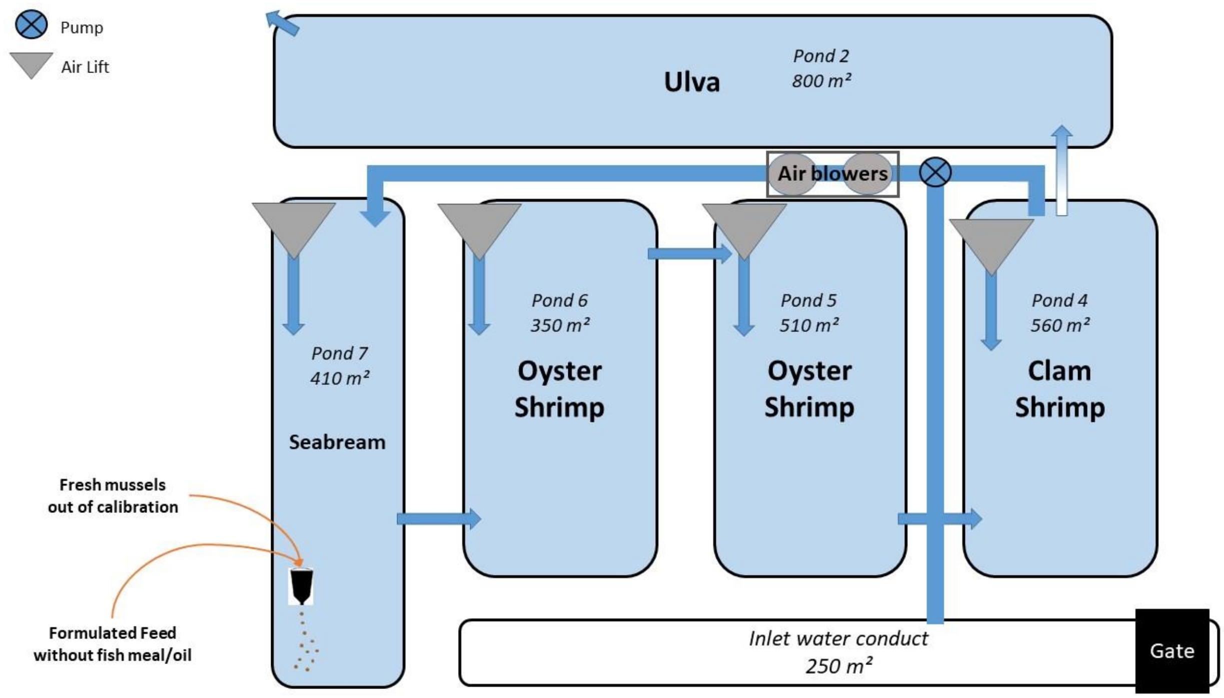


Fig. 27. Representation of the SIMTAP system developed in France.

The system was composed of 4 ponds, connected in cascade, to circulate the water by gravity, according to the following order:

- pond 7: 1387 seabreams were stocked at a mean weight of 210 g;
- pond 6: 700 oysters (*Crassostrea gigas*) and 875 shrimps (*Penaeus japonicus*) were stocked at a mean weight of 47.5 g and 0.52 g, respectively.
- pond 5: 1020 oysters and 1275 shrimps were stocked at a mean weight of 47.5 g and 0.52 g, respectively;
- pond 4: 11765 clams (*Ruditapes decussatus*) and 1400 shrimps were stocked at a mean weight of 3.4 g and 0.52 g, respectively.

From the pond 4, water was sent back to the pond 7 thanks to a pump. Initially, the pond 2 was supposed to receive the discharge water from the system during renewal of water. The nutrients contained in the discharge water should have been used for macro-algae growth (Ulva). In fact, since salinity remained at an appropriate level in the system, water was added from the inlet conduct (connected to the open sea) only to compensate evapotranspiration, avoiding discharge water toward the pond 2 and the surrounding environment. Therefore, this pond was considered more as a control pond (as well as the inlet channel).

During the experiment, the fish growth was checked by sampling fish three times, the shrimp growth was checked once, and the bivalves were not controlled before the end. The water quality was recorded each week to control temperature, oxygen, pH, turbidity and salinity. This information were also collected punctually to check the quality of water in the fishpond when the temperature increased. Once a month, water was sampled in each pond to check the concentration in phytoplankton, nitrogen compounds and phosphorus compounds. In addition, two measurement and water sampling campaigns on 24hours were conducted to check the daily evolution of the parameters. The water concentration in oxygen was a key parameter of the system. Despite the air adduction, it was at low levels during night and morning in the fish pond, which postponed the feeding time to the afternoon (**Fig. 28**). The mean concentration in oxygen for the entire period of experiment was significantly ($p<0.05$) lower in the pond 7 (6.5 ± 1.8 mg/l, n=13) than in the ponds 5 (9.5 ± 2.6 mg/l, n=13) and 4 (9.8 ± 2.8 mg/l, n=13), and in the inlet water conduct (8.7 ± 1.4 mg/l, n=9). No others significant differences were observed between the other compartments ($p<0.05$).

On early July, on the basis of 24 hours control, the mean concentration in oxygen was significantly ($p<0.05$) lower in the pond 7 (5.8 ± 0.6 mg/l, n=13) than in the ponds 5 (7.4 ± 1.2 mg/l, n=13) and 2 (7.1 ± 0.5 mg/l, n=13). On late August, the mean concentration in oxygen was also significantly ($p<0.01$) lower in the pond 7 (5.4 ± 1.2 mg/l, n=13) than in the ponds 5 (10.5 ± 2.9 mg/l, n=13) and 4 (11.0 ± 3.5 mg/l, n=13). The amplitude of the daily variation in oxygen concentration increased according to time and to the chlorophyll concentration. An increase of the concentration in total chlorophyll was observed in the system during the cycle of production (from 2.5 µg/l in June to 60.6 µg/l in October) contrary to that in the inlet water conduct (assumed to be similar to the open sea), in which the concentration remained quite constant (between 5.1 µg/l in June and 11.0 µg/l in October).

No significant differences were observed ($p<0.05$) between compartments, in the average concentrations in N (N total, NH4, NO2 and NO3) and P (P total and PO4). Nevertheless, the level of the N total in the ponds of the system was higher compared to the control ponds (pond 2 and the inlet water channel) in which the N total remained almost stable**.** along the production cycle time.

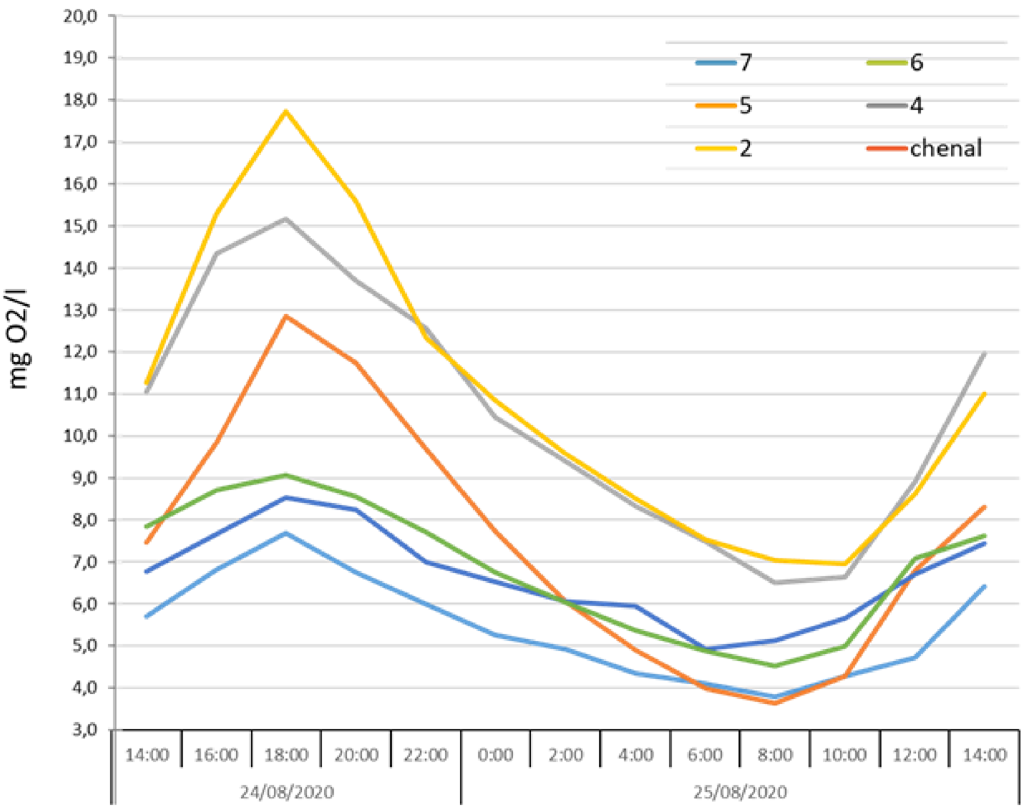


Fig. 28. Water concentration in dissolved oxygen in the different ponds during the 24h control in August 2020.

During the 24h control campaign, on late August, the means in N total, N-NH_4, N-NO_2, N-NO_3, P total and in the ponds from the system were significantly ($p<0.01$) higher than that in the control ponds (inlet water channel and pond 2), except for N-NO_3, P total in the pond 4 which were not different from the control ponds. The mean values of P-PO_4, were significantly lower ($p<0.05$) in all the ponds of the system than that in the control ponds.

Despite an adaptation period due to changes in feed and environmental conditions, seabreams reached the expected zootechnical performances (**Fig. 29**). 1 169 seabreams were harvested at a mean weight of 352.4 g, with an expected survival rate of 86.6%, and having a corrected FCR of 1.9.

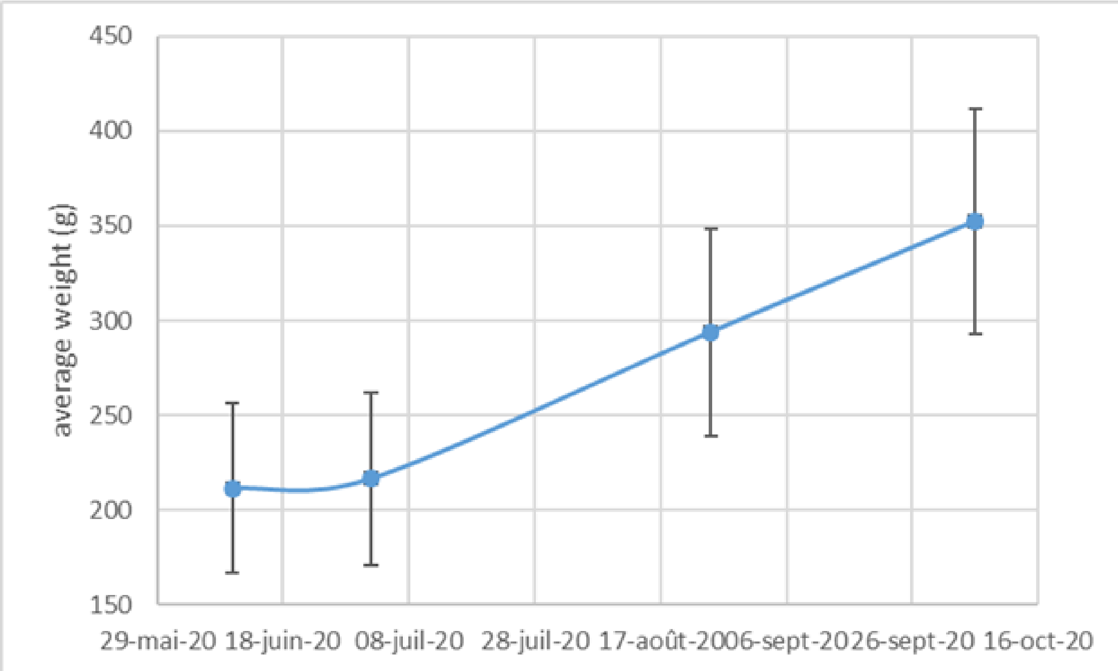


Fig. 29. Curve of seabreams average weight (with standard error) during the experimental period.

In two of the ponds the zootechnical performances of the shrimp reached the expected level. During the summer, the airlift from pond 6 (shrimp + oyster) was removed and set in the pond 7 to secure air supply in the fish pond. Unfortunately, the week before the harvest, a storm event depleted the oxygen in the water and all shrimps in the pond 6 died. Nevertheless, at the intermediate weighing, in late August, the mean weight of the shrimps from the pond 6 (24.1 ± 8.5 g, n=43) was significantly ($p<0.01$) higher than that in the ponds 5 (19.6 ± 8.3 g, n=34) and 4 (19.4 ± 8.8 g, n=31), and as heavy as the mean weight of the shrimps from the pond 5 (27.7 ± 11.9 g, n=118) weighed at the harvest time. At the harvest, the mean weight of the shrimps from the pond 4 was significantly ($p<0.01$) higher (35.2 ± 10.0 g, n=120) than that from the pond 5. The high weight variation is due to high sexual dimorphism.

The rearing performances of oyster were higher than expected. Despite summer mortality syndrome observed in the area, the survival rate observed oysters was 91% for pond 4 and 87% for pond 5. At the harvest, the mean weight of the oysters was significantly ($p<0.01$) better in the pond 5 (88.4 ± 20.6 g, n=89) than in the pond 6 (71.2 ± 10.4 g, n=89), as well as the mean filling ratio (21.5 ± 3.3 %, n= 50 and 18.8 ± 2.5 %, n=50, respectively). These filling ratios overpass the quality standard of Marenne-Oléron.

The number of clams harvested was estimated to 8 102, representing a survival rate of 69%, for a mean weight of 11.4 ± 3.3 g (n=100). Harvested clams had a filling ration of 20.0 ± 3.6% (n=50) which was significantly ($p<0.01$) higher than that measured in June (16.1 ± 3.0%, n=20).

In conclusion, in the system tested, good results were obtained for fish growth and feed conversion ratio, despite the lack of fish meal and fish oil in the feed. Without additional specific source of nutrients in the system, except than the feed supplied to the sea bream, the body growth of the others organisms reared were similar and even better (more specifically the filling ratio and survival rate of the mollusks) compared to their usual monoculture in ponds (based on expert experience). Even if the shrimps from pond 6 died, the intermediate weighing let expect an interesting growth potential from shrimps in this pond. Variations in the performances of the different species among the ponds give interesting perspectives in the improvement of the system. The different concentrations in N and P and physical indicators in water, like oxygen concentration, observed in the pond 4, reflected the ability of the system to improve the quality of the water released from the pond in which seabream were reared. Since water was added in the system only to compensate evaporation and no water was discharged during the study, it resulted in the save of water and in the preservation of the surrounding environment.

Work in progress

2.2.2. Studies on fish growth performances at fattening stage in SIMTAP system in Turkey: To compare the effectiveness of SIMTAP system in terms of fish growth, we used a flow through system as control. Both systems had the same tanks with about 5 tonnes (4 tonnes water holding capacity). Tanks were stocked with fish at the similar stocking densities. Fish were fed at predetermined levels based on the total biomass in each tank twice a day. Following the first fish stocking, all fish died due to electricity cut. On 24 August 2020, new set of fish were stocked to tanks once again and the experiment was continuing with these fish. Growth and specific growth rate (SGR) of fish maintained on the control and SIMTAP system are shown in **Fig. 30**.

Task 2.3: Assessing and optimizing the energy efficiency. Months (from 9 to 35). Task Leader: UNIBO **Participants:** UNIPI; INRA UMR SAS; LML; MAFA; MEDFRI

Work performed and main results

Due to the COVID restrictions – related in particular to delays in material deliveries and travel limitations – the Task 2.3 has experienced a remarkable delay. However, a few activities could have been carried out despite the restrictions. The work completed in the WP1, in particular the SIMTAP installations, the ISMaCS tests and the server availability, allowed to create informatics routines and codes to handle the collected data. Moreover, an analysis of the scientific literature allowed to identify possible methods to apply for an accurate data analysis.

Work in progress

Currently, UNIBO is assembling the ISMaCS and organizing their implementation on the experimental installations according to the pandemic restrictions and is testing and evaluating possible methodologies to be applied for the energy efficiency assessment.

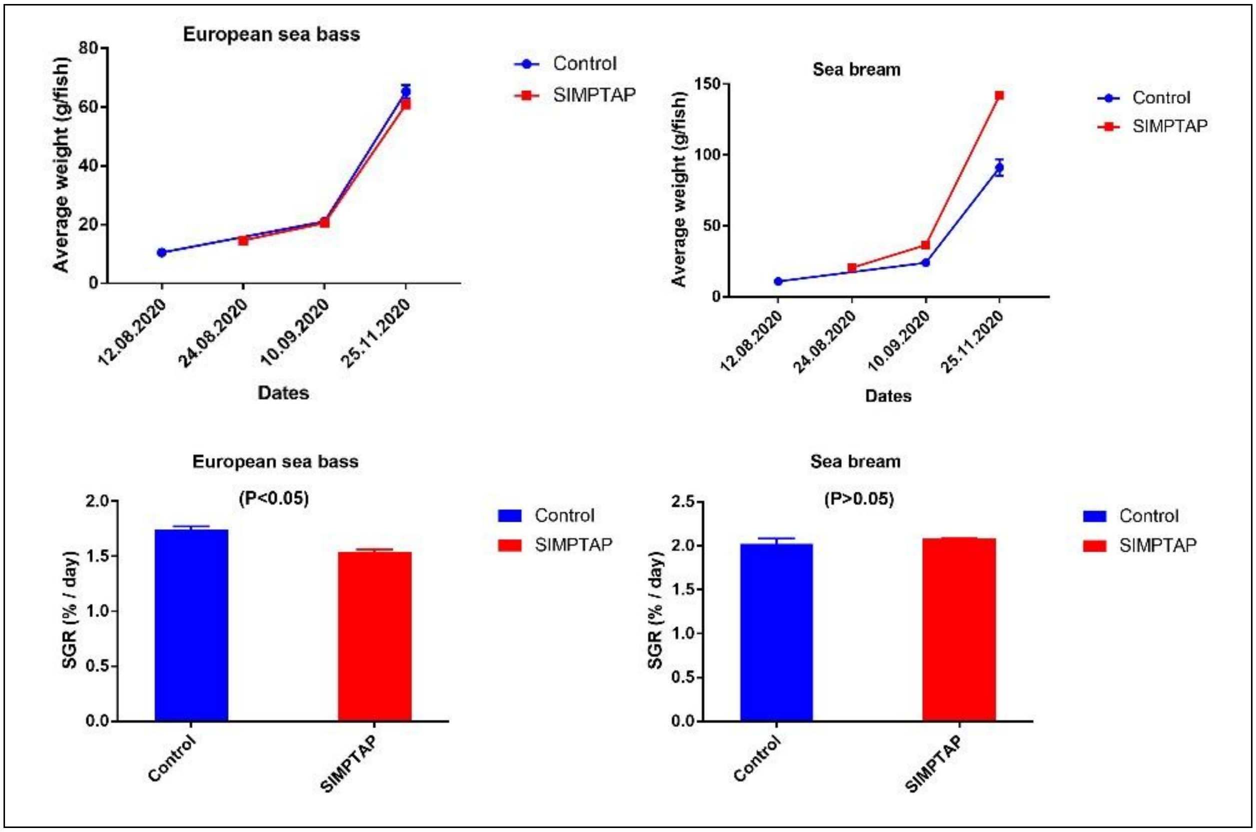


Fig. 30. Growth and SGR of European sea bass and gilthead sea bream reared in flow through and SIMTAP system.

Deliverables

Code	Title	Delivery date MONTH	Partner	Dissemination level (PU, CO)	Type	Status (completed, delayed, in progress)	Reason for the delay
D2.01	Report on system starting and biofilter conditioning	18	MEDFRI	CO	Report	Completed	
D2.02	Report on dietary inclusion levels of polychaetes and/or shellfish	24	MEDFRI, UNIPI	CO	Report	In Progress	
D2.03	Report and protocol on microalgae, polychaetes, shellfish and halophyte plant production and culture in SIMTAP system	31	MEDFRI, UNIPI, INRAE, LML, MAFA	CO	Report	In Progress	
D2.04	Report on growth performances and welfare of European sea bass, gilthead sea bream and mullet during early growth stages	32	MEDFRI, UNIPI, INRAE, LML	CO	Report	In Progress	
D2.05	Report on water and mass balance modelling	33	MEDFRI	R, CO	Report	in progress	
D2.06	Report on growth performances and welfare of European sea bass, gilthead sea bream and mullet during fattening stage	30	MEDFRI, UNIPI, INRAE, LML, MAFA	R, CO	Report	in progress	
D2.07	Report about the assessment of solutions tested in WP1	30	UNIBO	R, CO	Report	in progress	

Outputs

- Characterization of a native *Chlorella*-like microalgal strain isolated from a municipal landfill leachate and used in the SIMTAP photobioreactors.
- Three MSc thesis (Barbara Bassotti, Antonio Bindi and Alessandro Boni) on seawater hydroponic cultivation of salt-tolerant glycophytic and halophytic crop species. The dissertations were defended at the University of Pisa in 2020.
- An MSc thesis (Yasir Akbaş) on the evaluation of the nutritional value of polychaete meal for European sea bass (*Dicentrarachus labrax*). The thesis will be defended at the Akdeniz University in June (2021).

1.2.4 Work Package 3

Title: Integration of SIMTAP in current hydroponic systems to enhance market transferability and sustainability.

WP Leader: UNIBO

WP Partecipants: UNIBO, UNIPI, MAFA

Start month: 25 **End month: 30** (the end of this WP was postponed to M35)

Objectives: The aim of WP3 is the study of integration of SIMTAP solutions in current hydroponic systems to enhance market transferability and sustainability. Specifically, some open-loop hydroponic soilless systems, available in the market, will be considered and their run-off water from greenhouse soilless culture will be used as input for culture of both algae and halophytes in experiments conducted by UNIPI and MAFA. The development of a decision support system (DSS) aimed at defining the optimal location of SIMTAP systems based on multi-criteria GIS models will be also developed by UNIBO with the support of UNIPI

Five deliverables are foreseen for this WP. As reported in the proposal, the activities of WP3 will start on month 25

1.2.5 Work Package 4

Title: Assessing the quality of the food end-products

WP Leader: UNIPI

WP Participants: MEDFRI, LML, MESDC

Start month: 16 **End month:** 33

Objectives:

WP0 aims to assess the quality of the food products (plants and fish) in SIMTAP systems.

Two deliverables are foreseen for this WP, at month 33.

Work performed and main results

UNIPI

In the experiments with glasswort (*S. europaea*) grown in closed-hydroponic system with fresh water or artificial sea water containing 10 mM of N-NO_3 (Task 2.1), the content of chlorophylls, carotenoids and phenolics, and the total antioxidant capacity (FRAP assay) were measured in the edible shoots soon after harvest. Leaf pigments, such as chlorophyll and carotenoids, and phenolics are among the natural antioxidants in plants, which protect consumers against several diseases like cardiovascular diseases and cancer. Determination of the antioxidant activity in plant-derived foods is carried out using different methods, for instance by measuring the ferric-reducing antioxidant power (FRAP; it is the ability of plant tissues to reduce Fe(III)), the method used in this experiment. The higher the FRAP value, the higher the antioxidant capacity of the product.

The content of nitrates and sodium was also determined as they both may have toxic effects on humans. The European regulation 1258/2011 has set maximum limits for nitrates in some vegetable species such as spinach, rocket salad and lettuce. These limits range between 2000 and 7000 mg/kg FW depending on plant species, growing season and environment (Bian et al., 2020). They are higher for vegetables grown in fall-winter season and under greenhouse than in spring-summer and in the open field. Due to the well-known relationship between health outcomes and sodium intake, a daily intake of 2 g/day is considered safe for adult people by European Food Safety Authority (EFSA).

In glasswort, the content of the determined natural antioxidants (Tab. X). was in the range of values measured recently in sweet basil and lettuce grown hydroponically under greenhouse in very similar conditions to those of the experiments with glasswort (Puccinelli et al., 2021). As expected, shoot nitrate content was much higher in winter than in summer in the control (freshwater nutrient solution) and, in both seasons, it was markedly reduced by the use of sea water. The opposite results were found for sodium content, which was higher in summer than in winter in the plant grown in sea water and was greatly increased by the addition of IC to the nutrient solution. Based on the allowed daily intake (ADI) for both nitrates and sodium, the daily intake of fresh shoots (g FW per adult person) to reach ADI of nitrates (259 mg/adult per day) and sodium was calculated (**Tab. 11**). A safe daily consumption of glasswort shoots is 52 g in winter and 111 g in summer.

In a previous experiment conducted in spring two seawater nutrient solutions containing 10 mM or 1 mM N-NO_3 were tested. The lower N-NO_3 (corresponding to 62 mg/L of nitrates) was selected as it is close to the lower end of the range of nitrate concentrations in recirculating aquaculture system for *Sparus aurata* (the fish species raired in the SIMTAP system) reported by Tal et al. (2009). The nitrate and sodium content of the glasswort shoots was, respectively, 3659 mg/kg FW and 15.3 g/kg FW, in the high-N treatment, and 3082 mg/kg FW and 15.4 g/kg FW in the low-N treatment, with significant ($P<0.05$) differences between the two treatment only for shoot nitrate content. In this experiment, the calculated safe daily intake of fresh shoots was 130 g.

Tab. 11. Some nutritional traits of glasswort plants grown in closed-loop hydroponic systems under greenhouse in winter or summer 2020. The nutrient solution was prepared with fresh water or artificial sea water (d adding 30 g/L of the synthetic sea salt Instant Ocean, IC, were added to fresh water). The ADI of nitrates and sodium in adults is 289 mg and 2 g, respectively.

Parameter	**Winter**		**Summer**	
	Fresh water	**Sea water (30 g/L IC*)**	**Fresh water**	**Sea water (30 g/L IC)**
Chlorophylls content (mg/kg FW)	1650 a	644 b	474 c	687 b
Carotenoids content (mg/kg FW)	250 a	203 a	100 b	94 b
Phenols content (mg/kg FW)	2500 a	1801 a	2300 a	2805 a
FRAP (mmol/kg FW)	4.5 c	3.2 c	47.0 b	57.2 a
Nitrates content (mg/kg FW)	10750 a	1185 c	3410 b	1210 c
Daily intake of fresh shoots (g FW per adult person) to reach ADI of nitrates	24.1	218.6	76.0	214.0
Sodium content (g/kg FW)	3.9 c	38.0 a	4.7 c	18.0 b
Daily intake of fresh shoots (g FW per adult person) to reach ADI of sodium	512.8	52.6	425.5	111.4

Work in progress

UNIPI

In the long-term experiment conducted in 2021 to test the SIMTAP system installed at the University of Pisa, the quality of the fish and edible halophytes will be assessed, notably through the determination of the following quantities, according to the EU Regulation No. 1169/2011 on the provision of food information to consumers (https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv%3AOJ.L_.2011.304.01.0018.01.ENG&toc=OJ%3AL%3A2011%3A304%3ATOC): i) energy value; ii) the amounts of fat, saturates (saturated fats), carbohydrates, sugars, proteins and salt (sodium). The energy value and the amount of nutrients will be expressed per 100 g of product as such.

MEDFRI

A study on fish growth performances at the fattening stage in SIMTAP system in Turkey will be completed at the end of January (2021). Laboratory studies on fish fillet quality and nutritional values will be carried out within a few months. The results will be shared up to June (2021).

Studies on halophyte crop production will be carried out between March and August (2021). The results on nutritional value and quality of halophyte plants will be shared up to December (2021).

Deliverables

Code	Title	Delivery date (month)	Responsible	Dissemination level	Type	Status (completed; in progress; delayed)
D4.02	Report on nutritional value and quality of halophyte plants	M33	UNIPI	CO	Report	In progress
D4.01	Report on fish fillet quality and safety and nutritional values	M33	UNIPI	CO	Report	In progress

Outputs

The MSc thesis of three students at the University of Pisa reports some experiments conducted on salt-tolerant glycophytic or halophytic species grown in hydroponic systems with seawater.

1.2.6 Work Package 5

Title: Economic, environmental and social sustainability assessment
WP Leader: INRAE

WP Participants: ALL

Start month: M1 End month: M36

Objectives:

The general objective of WP5 is to build an overall assessment of sustainability of the SIMTAP systems compared to standard systems. The methodology is based on Life Cycle Assessment (LCA) for environmental goals, Emergy accounting (EA) for energetic and sustainable goals, Life Cycle Costing (LCC) for economic goals, and Social LCA (SLCA) for social goals. In order to build an overview of the sustainability, these indicators as well as additional indicators of system efficiency are organized in a qualitative decision tree permitting to aggregate the indicators into principles of sustainability, using a participatory method (DEXI method). The responsibility of LCA, EA, and DEXI methods was carried out by INRAE and the responsibility of the LCC and SLCA was carried out by UNIMI. The both teams worked closely together on the different tasks, and involved all SIMTAP partners in the participatory process.

The operational objectives are: i) to implement tools such as economic models (e.g. LCC) and logic framework project planning concept that can assist farmers to improve their management and secure a sustainable income; ii) To identify the environmental performances of the whole integrated cycle respect to aquaculture + hydroponic and the main hotspots (processes mainly responsible of this impact) using the Life Cycle Assessment (LCA) approach and Emergy analysis. LCA and other energy and resource evaluation will be also done, and the results obtained will be compared thanks to a qualitative approach (decision trees DEXi software); hence the most suitable species and rearing conditions will be defined.

Eighteen deliverables are foreseen for WP5.

Task 5.1 Global multicriteria assessment approach (M1-M6; M26-M36) Task leader: INRA/Partners involved: UNIMI, MEDFRI.

Work performed and main results

The task 5.1 of the work package 5 aims to develop a global approach to assess sustainability of SIMTAP systems, in order to organize the different indicators resulting from environmental impacts assessment (Life Cycle Assessment and Emergy Assessment) and socio-economic impacts assessment (Social Life Cycle Assessment and Life Cycle Costing). A qualitative approach based on decision trees has been be carried out, applying a decision support system software called DEXi (https://kt.ijs.si/MarkoBohanec/dexi.html) stemmed from DEX methodology (Bohanec, 2003).

DEX for Decision Expert is a methodology that combines hierarchical multi-attribute model decision making with an expert systems approach based on qualitative attributes or also called criteria (Bohanec 2003; Craheix et al. 2015). All the attributes in the model are qualitative and represented by words (low, medium, high). DEXi allows the breakdown of a complex decision problem into smaller and less complex sub-problems (Bohanec, 2003; Craheix et al., 2015). Mobilizing a large reference literature as well as interviews of specialists, a wide list of principles, criteria and indicators of sustainability was proposed. According to the Dexi method, this list was prioritized and a decision tree linking all the different concepts was built, involving the different partners of the project and external advises. The different concepts were then weighted using a participatory process, to obtain the final DEXI tree. Different steps were achieved in order to conduct the application of Dexi method to Simtap project, as presented in **Fig. 31.**

All the process of construction the Dexi-aqua tree has been conducted up to the end. Therefore, different products have been obtained:

- A list of relevant and operational indicators for the assessment of the three pillars of the sustainable development (including those from LCA, LCC, SLCA and Emergy Accounting) adapted to aquaculture, with their thresholds values,
- Three decision trees (one by sustainable pillar), organising the indicators and their aggregation and weighting.
- A template for the data collection and indicators calculation
- A guide to use the different tools.

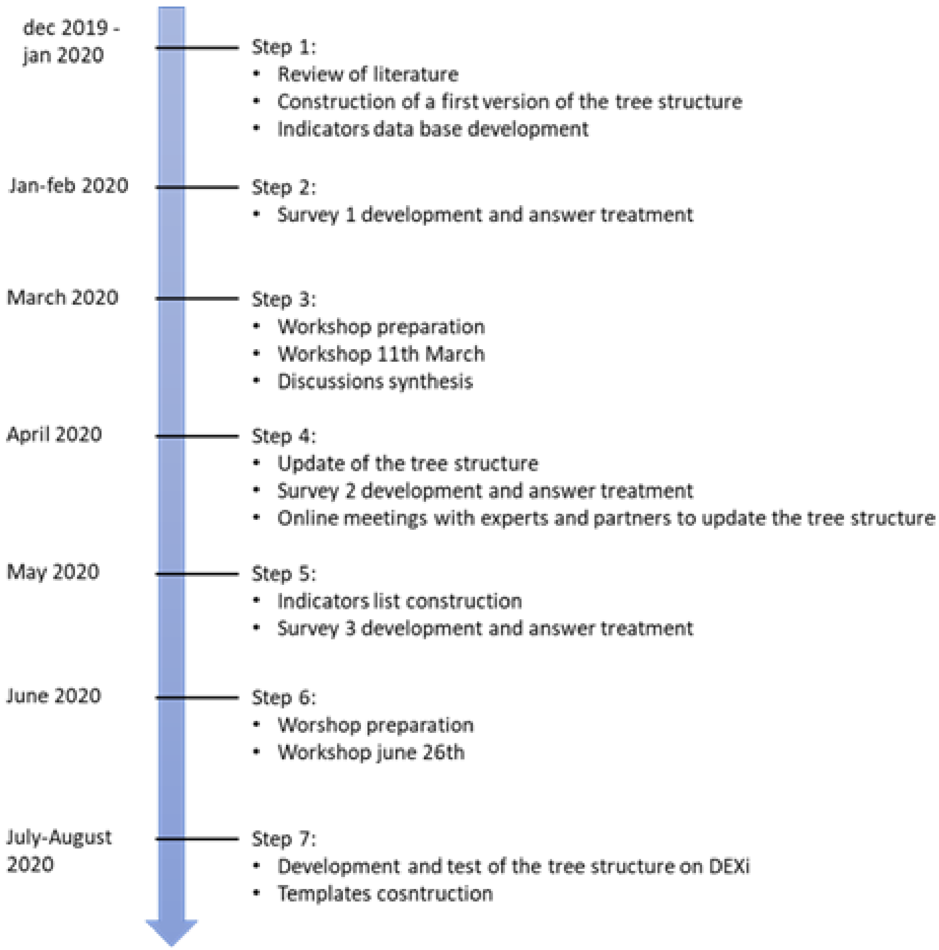


Fig. 31: Dexi-aqua method chronology.

Work in progress

The next step will consist in the validation, and adaptation of the Dexi-aqua trees, to the different production contexts. To do so, the assessment of reference systems to fix the last remaining points and generate reference values for the sustainability assessment of the SIMTAP new systems, will be perform by the project partners.

Task 5.2 Identifying the economic performances (M1-M6; M22-M36) Task leader: UNIMI/Partners involved: INRA, KOROLEV, MEDFRI.

Work performed and main results

Life-cycle analysis (LCA) does not account for economic aspects, and such analysis should therefore be considered together with a life-cycle cost analysis (LCC), which takes into account the costs of investment, energy, maintenance, and dumping the final waste product throughout the lifetime of a plant (Swarr et al, 2011).

Life-cycle cost analysis (LCC) is an approach for evaluating all relevant costs over time of a project, product, or services. It takes into consideration all costs including first costs, such as capital investment costs, purchase, and installation costs; future costs, such as energy costs, operating costs, maintenance costs, capital replacement costs, financing costs; and any resale, salvage, or disposal cost, over the lifetime of the project or product. LCC is thus an engineering economic analysis approach useful for comparing the relative merit of competing project alternatives. LCC is not standardized like environmental LCA and many different conceptual frameworks to perform this analysis have been proposed in literature but the most common to date has been the use of cash flow models (Falcone et al., 2016). The latter approach was therefore selected to evaluate the economic performance of SIMTAP systems and to critically compare these with commercial aquaculture plants.

In this task, regarding the Life Cycle Cost (LCC), to assess the economic performances from each SIMTAP system under investigation:

- A data collection form has been developed by for the gathering of the information about the different production factors (e.g. amount, specific costs) and the capital goods (investment, life span) as well as about the productive performances of the different SIMPTAP systems under evaluation.
- The data collection form has been shared with the partners involved in WP2, WP3 and WP4. Contacts were established with some aquaculture plants located in the south of Tuscany and the list of economic data needed for the assessment of seabream and seabass production in inland aquaculture plants was discussed with plant managers.
- An Excel-based tool for the calculation, for the different SIMTAP systems, of NPV (Net Present Value), Internal Rate of Return (IRR) and Gross Added Value (GAV) was set-up

Work in progress

The next steps will consist in: (i) the collection, in cooperation with the partners, of the inventory data for LCC analysis for commercial aquaculture plants and for SIMTAP systems; (ii) processing of the collected data and analysis of the economic performances

Task 5.3 Identifying the environmental performances (M1-M6; M22-M36) Task leader: INRA/Partners involved: UNIMI, MEDFRI.

Work performed and main results

In this task, the Life Cycle Assessment (LCA) is the methodology selected to assess environmental impacts from each SIMTAP system studied. LCA is a standardized framework (ISO, 2006a; b) designed to estimate potential impacts associated with producing a product by quantifying and estimating resources consumed and compounds emitted into the environment during all stages of its life cycle, from raw material extraction up to the end-of-life (Guinée et al. 2002). Each substance emitted or consumed is assigned to one or more impact categories based on its potential environmental impact and information from scientific literature. The LCA performed in this study followed the main guidelines in the ILCD handbook (Joint Research Centre 2010). It is composed of four main steps: Goal and scope definition, Life Cycle Inventory (LCI), Life Cycle Impact Assessment, Results analysis. At this stage the work was focussed on the two first steps. The LCI template has been integrated to Dexi template since indicators from LCA are used in Dexi, to make data collection easier. A workshop was carried on October 9th, 2020 to present to all the partners the LCA methodology and the information expected to fill the LCI template.

A LCI template, adapted to SIMTAP systems, has been established.

Work in progress

The LCI template was sent to all partners who will fill it before the end of February 2021.

Task 5.4 Emergy accounting (M1-M6; M22-M36) Task leader: INRA

Work performed and main results

Here, the Emergy Accounting (EA) method was chosen in addition to LCA methodology to further assess environmental impacts from each studied SIMTAP system. EA (Odum and Odum,2003;) is a tool based on Energy Systems Theory (Odum, 1983), which was developed to integrate all system inputs (i.e. resources, services and commodities) using a common unit. Prior to carrying out the assessment, an inventory template has been established, gathering information on all the elements (inputs from nature and technosphere and outputs) involved in the production of all the products from each studied system. The EA template has been integrated to the global Dexi template to make data collection easier for the partners.

A dedicated workshop was carried on October 9th, 2020 to present to all the partners the environmental and sustainability assessment methodology (ie including EA, LCA and DEXI methods) and the information expected to fill the template.

An EA template integrated to the global DEXI template has been established. This template was specifically designed to fit SIMTAP systems,

An internal course on LCA and Emergy accounting was held for the project partners.

The global template has been forward to all partners who are expected to fill it before the end of February 2021.

Task 5.5 Identifying the social performances (M1-M6; M22-M36) Task leader: UNIMI/Partner involved: MEDFRI.

Work performed and main results

The aim of this task is to assess the social sustainability contributing to the full assessment of the SIMTAP systems. Social sustainability is one of the three pillars of sustainability also included within the global multicriteria assessment framework (Task 5.1). Unlike the latter, for this specific task the social results will be presented separately to better highlight the societal risks and benefits of the SIMTAP system. However, the inventory data needed for the two analyses (global multicriteria assessment and social life cycle assessment) concur. Therefore, the stakeholders, social themes and sub-categories relevant to the project from the social point of view were selected under the guidance of INRAE, also through surveys and frequent discussione (via email, video calls and on-line workshops) with the project members.This selection was made following the principles dictated by the UNEP (United Nations Environment Programme) Guidelines for SLCA (Guidelines for Social Life Cycle Assessment of Products), which have been adapted to the system under evaluation, thus inserting sub-categories of specific interest for aquaculture production while excluding some others considered not of interest. Starting from this, threshold values were attributed to each sub-category, based on scientific literature and experts' opinion, for their evaluation through performance references points. The data collection form has been developed for gathering information about the social relevant aspects of the different SIMTAP systems under evaluation. The data collection form has been shared with the partners involved in WP2, WP3 and WP4. Contacts were established with some aquaculture farms located in the south of Tuscany and the list of social figures needed for the assessment of seabream and seabass production in inland aquaculture plants was discussed with farm managers.

Work in progress

The next steps will consist in: i) definition of a method for aggregating and weighing the inventory data to better express the social results, in order to highlight the social hotspots and to compare in depth the SIMTAP systems and those with conventional production cycles. To do this, the aggregation and weighing methods proposed by the UNEP Guidelines will also be considered. These are currently under review and the updated version will be published at the end of January 2021. Since then they will be analyzed to verify if there have been methodological updates for the assessment of the social impact of agri-food processes and finally the aggregation methods will be confirmed; (ii) the collection, in cooperation with the partners, of the inventory data for social analysis for commercial aquaculture plants and for SIMTAP systems; (iii) social performance analysis, in order to identify the critical social aspects of a IMTA aquaculture management and to find ways of improve them in future in order to trace the path for a socially sustainable aquaculture, also through a direct comparison with aquaculture farms managing the conventional production cycle.

Deliverables

Code	Title	Responsible	Due date (month)	Status (completed; in progress; delayed)	Reasons for the delay
D5.01	General report on methodology and definition of Baseline Scenario	INRAE	2019-12-01	Completed	
D5.02	LCC template and survey design	UNIMI	7	Completed	
D5.03	LCI template	INRAE	7	Completed	
D5.04	Emergy template (common with LCA)	INRAE	7	Completed	
D5.05	Social assessment template	UNIMI	7	Completed	
D5.06	Internal report about the subsidy possibilities	KOROLEV	26		
D5.07	LCC inventory	UNIMI	30		
D5.08	Progress report on energy efficiency	UNIBO	30		
D5.09	LCI database	INRAE	30	In progress	
D5.10	Emergy inventory	INRAE	30	In progress	
D5.11	Social assessment database	UNIMI	30		
D5.12	LCC results	UNIMI	34		
D5.13	LCA results	INRAE	34		
D5.14	Emergy results	INRAE	34		
D5.15	Social assessment	UNIMI	34		
D5.16	General report on multicriteria performances of SIMTAP in different contexts	INRAE	36		
D5.17	Report for energy efficiency	UNIBO	36		
D5.18	Internal report on environmental assessment	INRAE	36		

Outputs

- Dexi-Aqua methodology report
- Dexi-Aqua sustainable indicators report
- Dexi- template
- Data collection template guidelines
- Data collection form for the collection of the economic information,
- Excel-based tool for the LCC analysis
- Guidelines for the data collection and for the use of the developed tool
- LCI template and data collection guidelines integrated to documents related to Dexi.
- Emergy Accounting template and data collection guidelines integrated to documents related to Dexi modeling.
- List of stakeholder type, social subcategories that will be considered
- Data collection form for the collection of the social information.

Attachments

- Dexi tool: templates; data collection template guidelines; Dexi-Aqua methodology report; Dexi-Aqua sustainable indicators report.

1.2.7 Work Package 6

Title: Assessing the quality of the food end-products

WP Leader: UNIMI

WP Participants: ALL

Start month: 18 **End month:** 33

Objectives:

WP0 aims to assess the quality of the food products (plants and fish) in SIMTAP systems.

Seven deliverables are foreseen for this WP.

The work has started recently and there are still no important results to include in this report.

1.2.8 Work Package 7

Title: Communication, dissemination and exploitation.

WP Leader: UNIBO

WP Participants: ALL

Start month: 1 **End month:** 36

Objectives: WP7 aims principally at i) creating community awareness and raising awareness of the project developments to different categories of stakeholders through an effective project communication, ii) performing training actions for different stakeholders, III) Ensuring the best possible foundation for an appropriate exploitation of the project results, and iii) disseminating the project results to the scientific community, stakeholders in the sector of aquaculture and food production and the general public.

Ten deliverables are foreseen in this WP.

Task 7.1 Communication activities (M1-M36) Task leader: UNIBO/Partners involved: ALL.
A detailed Communication Plan was written. The communication plan (CP) aims at enhancing the visibility of SIMTAP project and arising public awareness on the research goals and expected results. Project consortium members ensure that the project is and will be adequately promoted through the following different means and through the involvement of adequate media experts.

The communication started with the kick-off meeting of the project and has been carried on with activities that took place during the whole project period. Unfortunately, despite the project officially started on June 1, 2019 and the kick-off meeting was held on June 26-27 in Pisa, due to the long and complex procedure envisaged by the MUR (Italian Ministry for University and Research) for the funding of international research projects, the research agreement (compulsory act) between the same MUR and the three Italian universities was not signed yet. Consequently, no funding was disbursed and only the UNIPI and UNIBO partners received a partial advance of the funds from their own university. All the other Italian partners of the projects approved in 2018 (around 20) are in the same condition as UNIPI, while all the foreign partners of the SIMTAP project and of the other PRIMA 2018 projects have signed research contracts with national funding bodies. Nevertheless, besides the Covid-19 pandemic, few results were accomplished to be communicated in newsletter.

A common public image/branding for SIMTAP allows an easier identification by the public and ensures visibility and recognition. Therefore, project logo and common graphics for the project template (e.g., presentation, template, report, etc.) and any published or publicly presented material (e.g., brochures, leaflets, flyers, posters, etc.) has been created. The selected logo is:

Moreover, an institutional presentation template, containing basic information about the SIMTAP project has been developed, to act as a basis for relevant communication activities. The standard SIMTAP presentation may be used by the project consortium for dissemination purposes at relevant events, according to the type and size of audience/events where the project will be presented and be also regularly updated in the future. A template for public deliverables and reports was designed, remembering that any communication activity must duly display the EU emblem, the PRIMA logo and the following acknowledgment: "[SIMTAP/grant code] is part of the PRIMA Programme supported by the European Union".

The Website for the SIMTAP Project was created and it is still updated every few weeks and at every important even. The site is visible at the following url: https://www.simtap.eu/index.php

SIMTAP Project profiles have been created also on Social Networks as follows:

- Facebook: https://www.facebook.com/SimtapProject
- Twitter: https://twitter.com/ProjectSimtap
- YouTube: https://www.youtube.com/channel/UC2olupsbww4iynNop-RgH_Q
- ResearchGate: https://www.researchgate.net/project/SIMTAP-Self-sufficient-Integrated-Multi-Trophic-AquaPonic-systems-for-improving-food-production-sustainability-and-brackish-water-use-and-recycling

At the beginning of the project, TV broadcastings were done and they can be watched at the following Web sites:

- http://futuro24.blog.rainews.it/2019/05/31/futuro24-tecnologie-per-unagricoltura-sostenibile/
- https://primaobservatory.unisi.it/en/projects/simtap-self-sufficient-integrated-multi-trophic-aquaponic-systems-for-improving-food-production-sustainability-and-brackish-water-use-and-recycling
- https://www.youtube.com/watch?v=umHouF6tsKc&feature=emb_logo

Task 7.2 Dissemination activities (M1-M36) Task leader: UNIPI /Partners involved: all

Project consortium members ensure that project results are adequately disseminated through different means:

- publication of joint scientific papers on peer reviewed journals using Gold and/or Green Open Access approach. At the moment, three papers were published, one was accepted and other manuscripts are in progress.
- Participation of consortium members in events where the research performed can be presented. Due to the Covid-19 pandemic situation only very few on-line events were organized after the kick-off meeting:
    - BRIGHT-NIGHT "Brilliant Researchers Impact on Growth Health and Trust in research" at UNIBO. https://www.nottedeiricercatori.it/

- IEEE MetroAgriFor 2020 Workshop. Virtual Conference | 4-6, November 2020. https://ieeexplore.ieee.org/xpl/conhome/9276490/proceeding
- International Conference on Environmental Science & Technology, 4-7 September 2019, Rhodes, Greece. https://cest2019.gnest.org/proceedings

– Issuing of a regular newsletter (every six months) collecting the project results. It will be sent to the scientific community (e.g., national and international associations of agricultural and biosystem engineering, EurAgEng).

  The newsletter has been implemented through the website and social media. To increase the visibility and efficacy, the news feeding procedure within the consortium and dissemination to the stakeholders has been enhanced assuring a broad participation of all partners. Moreover, a constant research to widen the audience has running since the beginning of the activities and is planned to run throughout all the project.

– Training sessions for stakeholders, which will be organized towards the end of the project.

– Liaison/collaboration with relevant European communities, EU projects and EU R&I funding initiatives.

  The SIMTAP project activities will be able to benefit of collaborations with the ERA-HDHL Knowledge Hub on Food and Nutrition Security SYSTEMIC, in particular in the WP2 that deals with "New developments in integrated food production systems", and specifically in Task 2.2 that has a specific focus on the aquaculture systems. A SIMTAP webinar in 2021 will be organized and members of SYSTEMIC will be invited and encouraged to give contributions and feedbacks.

Task 7.3 Exploitation planning and IPR management (M1-M36) Task leader: UNIPI /Partners involved: all

Following Art. 28 of the Model Grant Agreement, the exploitation activities will develop during the project. A solid plan for commercially exploiting the project results by the whole consortium or by one or partners was foreseen at month 7 (Del. 07.05); however, due to the delay of the project activities, it has been postponed to month 24, when an exploitation agreement /Del. 07.07) will be signed.

Deliverables

Code	Title	Delivery date (Month)	Partner	Dissemination level (PU, CO)	Type	Status	Reason for the delay
D7.01	Press releases	2	UNIPI	PU	News Item/Press	In progress	
D7.02	Communication Plan	2	UNIPI	CO	Working paper	Completed	
D7.03	Project website & Facebook, Twitter and LinkedIn accounts	2	UNIPI	CO	Website	Completed	
D7.04	Project's corporate identity set, presentation, promotional material, etc.	3	UNIPI	PU	Website	Completed	
D7.05	Plan for the dissemination and exploitation of results	~~7~~ 24 (7)	UNIBO	CO	Working paper	Delayed	Most activities of the project have been delayed by lack of funds and the pandemic Covid-19
D7.06	Electronic newsletters	24 (7)	UNIPI	PU	Newsletter	In progress	
D7.07	Exploitation agreement	24 (7)	UNIPI	CO	Working Paper	Delayed	
D7.08	Joint papers published	12 (onwards)	ALL	PU	Journal article	In progress	
D7.09	Project video	24	KOROLEV	PU	Video	-	
D7.10	2 workshop proceedings and 1 conference proceedings	36	ALL	PU	Conference Proceedings		

Publications:

- Bacenetti et al. (2019), *Approaches to tackle emerging challenges in European aquaculture*. 16th International Conference on Environmental Science & Technology, 4-7 September 2019, Rhodes, Greece.
- Barbaresi et al. (2020), *A Smart Monitoring System for Self-sufficient Integrated Multi-Trophic AquaPonic*. Proceedings of IEEE MetroAgriFor 2020 Workshop.
- Ciurli et al. (2021), *Multidisciplinary integrated characterization of a native Chlorella-like microalgal strain isolated from a municipal landfill leachate*. Algal Research (accepted).
- Rossi L. Bibbiani C., Fierro-Sañudo J.F., Chinoilema Maibam C., Incrocci L., Pardossi A., Fronte B. (2021). *Selection of marine fish for Integrated Multi-Trophic Aquaponic production in the Mediterranean area, using DEXi multi-criteria analysis as innovative approach.* Aquaculture, 2021, 736402. https://doi.org/10.1016/j.aquaculture.2021.736402.

1.3 Impact

The information on section 2.1 of the proposal submitted (how your project will contribute to the expected impacts) is still relevant or needs to be updated. Include further details in the latter case.

Expected impact	KPIs	Related Specific Objective
Design of public policies aimed at enhancing adoption of innovation suited to improve farmers' livelihoods.	Dissemination activities. Suggestions for public policies: regulations, promotion of producer associations, strategic planning, start-up policies, marketing policies, expansionary policies.	SO10
Implementation of tools (best practices, decision support system, models, discussion and co-development platforms) that can assist farmers to improve farm management in a risky and uncertain environment, and secure a sustainable income.	Dissemination events and materials. Training sessions.	SO4; SO10
Delivery of participatory approaches for integrating farmers' knowledge in the innovation process.	Stakeholders awareness of the SIMTAP project ; interaction activities with stakeholders; training actions for different stakeholders (Farmers, Producer Associations, Aquaculture Technologists)	SO10

Growth of rural employment and poverty alleviation	Number of created jobs 5 years after the end of the project: 600-650 direct jobs; 60-65 indirect jobs.	SO3, SO7, SO9
Contribute to a balanced territorial development		SO4, SO9, SO10
Transferability	Technical specifications; prototypes in different countries; DSS.	SO1, SO2, SO4
Reduction of the environmental impact	LCA and emergy analysis	SO1, SO2, SO3, SO6, SO8
Lower volatile production costs and stable profitability	Cost and investment analysis	SO7
Reduction of dependence on international markets		SO5, SO6, SO7, SO9

2. Update of the plan for exploitation and dissemination of result (if applicable)

Due to the delay of much of the project activities, the plan for the dissemination and exploitation of results (Del. D7.05) and the exploitation agreement (Del. D707) have not yet prepared or achieved. The new deadline for this achievement is the end of May 2020 (M24).

3. Update of the data management plan (if applicable)

Not applicable

4. Follow-up of recommendations and comments from previous review(s) (if applicable)

Not applicable

5. Deviations from the proposal submitted (if applicable)

The workplan reported in the proposal was modified because most of the partners are significantly behind the schedule due to problems with the NFB (in Italy and in Germany) and the pandemic.

According to the Consortium Agreement, the new workplan with extended duration of WP2-4 and the new schedule of some deliverables (see next page) was approved by the General Assembly, which met online on 19 November 2020.

	SIMTAP GANTT CHART	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24	M25	M26	M27	M28	M29	M30	M31	M32	M33	M34	M35	M36
WP0	SIMTAP coordination and management																																				
T0.1	Technical coordination																																				
T0.2	Financial and administrative management																																				
T0.3	Quality assurance																																				
WP1	Ecosystem based approach for SIMTAP							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E															
T1.1	Piloting activities							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E															
T1.2	Study of the most suitable plants							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E															
T1.3	Study of the dietary inclusion levels							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E															
T1.4	Designing, building and trying out an integrated smart monitoring and control system							E	E	E	E	E	E	E	E	E	E	E	E	E	E	E															
WP2	Implementation and test of SIMTAP																															E	E	E	E	E	
T2.1	Pilot tests																															E	E	E	E	E	
T2.2	Testing SIMTAP or its specific parts																															E	E	E	E	E	
T2.3	Assessing and optimizing the energy efficiency																															E	E	E	E	E	
WP3	Integration of SIMTAP in current hydroponic systems to enhance market transferability and sustainability																															E	E	E	E	E	
T3.1	Studying and testing the algae and feeders production cycles using brackish water...																															E	E	E	E	E	
T3.2	Evaluating the final run-off from the SIMTAP system																															E	E	E	E	E	
T3.3	Development of a decision support system aimed at defining the optimal location of SIMTAP systems...																															E	E	E	E	E	
WP4	Assessing the quality of the food end-products																																		E	E	
WP5	Economic and environmental sustainability assessment																																				
T5.1	Global multicriteria assessment approach																																				
T5.2	Identifying the economic performances																																				
T5.3	Identifying the environmental performances																																				
T5.4	Emergy accounting																																				
T5.5	Identifying the social performances																																				
WP6	SIMTAP recommendations and guidelines																																				
T6.1	Guidelines and best practices																																				
T6.2	User's manual for SIMTAP																																				
WP7	Communication, dissemination and exploitation																																				
T7.1	Communication activities																																				
T7.2	Dissemination activities																																				
T7.3	Exploitation planning and IPR management																																				
♦	Milestone																																				

The new project Gantt chart approved by the General Assembly of SIMTAP.

List of deliverables

Number	Title	WP	Delivery date (month)	New Delivery date (month)	Partner	Dissemination Level (PU or CO)	Deliverable type (Dropdown Menu)
D0.01	Project management tool (regularly kept updated)	0	1		UNIPI	PU	Tool
D0.02	Financial and administrative guidelines	0	2		UNIPI	PU	Working Paper
D0.03	Quality Assurance Plan	0	3		UNIPI	PU	Working Paper
D0.04	Project's reports (Periodic reports and Final Report)	0	36		ALL	PU	Report
D1.01	SIMTAP design	1	5		UNIPI (INRAE, MEDFRI, MAFA)	PU	Demonstrator, pilot, prototype, plan designs
D1.02	Review paper on integrated smart monitoring and control systems (ISMaCS) for agri-food productions	1	3		UNIBO	CO	Report
D1.03	ISMaCS design	1	5		UNIBO	PU	Demonstrator, pilot, prototype, plan designs
D1.04	SIMTAP prototypes in Italy, France, Turkey and Malta	1	6	21	UNIPI, INRAE, MEDFRI, MAFA	PU	Website
D1.05	Report on halophyte plant species for SIMTAP production	1	7		UNIPI	CO	Report
D1.06	Report on algae species for SIMTAP production	1	3		UNIPI	CO	Report
D1.07	Report on polychaetes and shellfish in a hypothetical SIMTAP system	1	7		UNIPI	CO	Report

Number	Title	WP	Delivery date (month)	New Delivery date (month)	Partner	Dissemination Level (PU or CO)	Deliverable type (Dropdown Menu)
D1.08	Building, calibration and test of ISMaCS	1	7	21	UNIBO	CO	Report
D1.09	Report on ISMaCS calibration and test: report on the ISMaCS functions and quantification of the reliability of the system	1	7	21	UNIBO	CO	Report
D2.01	Report on system starting and biofilter conditioning	2	10	18	MEDFRI	CO	Report
D2.02	Report on dietary inclusion levels of polychaetes and/or shellfish	2	18	24	MEDFRI	CO	Report
D2.03	Report and protocol on microalgae, polychaetes, shellfish and halophyte plant production and culture in SIMTAP system	2	28	31	MEDFRI	CO	Report
D2.04	Report on growth performances and welfare of European sea bass, gilthead sea bream and mullet during early growth stages	2	20	32	MEDFRI	CO	Report
D2.05	Report on water and mass balance modelling	2	26	33	MEDFRI	CO	Report
D2.06	Report on growth performances and welfare of European sea bass, gilthead sea bream and mullet during early growth stages	2	30		MEDFRI	CO	Report

Number	Title	WP	Delivery date (month)	New Delivery date (month)	Partner	Dissemination Level (PU or CO)	Deliverable type (Dropdown Menu)
D2.07	Report about the assessment of solutions tested in WP1	2	30		UNIBO	CO	Report
D3.1	Report on the use of brackish water and/or exhausted nutrient solutions to grow algae, and halophytes; obtained biomass productions	3	30		UNIPI	CO	Report
D3.2	Report on the polychaetes and deposit/filter feeders productions	3	30		UNIPI	CO	Report
D3.3	Report on the water run-off and the amount of brackish water for replacement. Report on the reduction of the run-off of the cascade system compared with a standard hydroponic system	3	30		UNIPI	CO	Report
D3.4	Geodatabase of multicriteria analysis results in the countries involved	3	30		UNIBO	PU	Dataset
D3.5	Decision support system developed in a GIS Environment	3	30		UNIBO	PU	Software
D4.01	Report on fish fillet quality and safety and nutritional values	4	33		UNIPI	CO	Report
D4.02	Report on nutritional value and quality of halophyte plants	4	33		UNIPI	CO	Report

Number	Title	WP	Delivery date (month)	New Delivery date (month)	Partner	Dissemination Level (PU or CO)	Deliverable type (Dropdown Menu)
D5.01	General report on methodology and definition of Baseline Scenario	5	7		INRAE	CO	Report
D5.02	LCC template and survey design	5	7		UNIMI	CO	Template
D5.03	LCI template	5	7		INRAE	CO	Template
D5.04	Emergy template (common with LCA)	5	7		INRAE	CO	Template
D5.05	Social assessment template	5	7		UNIMI	CO	Template
D5.06	Internal report about the subsidy possibilities	5	26		KOROLEV	CO	Report
D5.07	LCC inventory	5	26	30	UNIMI	CO	Dataset
D5.08	Progress report on energy efficiency	5	26	30	UNIBO	CO	Report
D5.09	LCI database	5	26	30	INRAE	CO	Dataset
D5.10	Emergy inventory	5	26	30	INRAE	CO	Dataset
D5.11	Social assessment database	5	26	30	UNIMI	CO	Dataset
D5.12	LCC results	5	30	34	UNIMI	CO	Report
D5.13	LCA results	5	30	34	INRAE	CO	Report
D5.14	Emergy results	5	30	34	INRAE	CO	Report
D5.15	Social assessment	5	30	34	UNIMI	CO	Report
D5.16	General report on multicriteria performances of SIMTAP in different contexts	5	36		INRAE	CO	Report
D5.17	Report for energy efficiency	5	36		UNIBO	CO	Report
D5.18	Internal report on environmental assessment	5	36		INRAE	CO	Report
D6.01	Review of the main technical, socio-economic and environmental findings	6	28		UNIMI	CO	Report

Number	Title	WP	Delivery date (month)	New Delivery date (month)	Partner	Dissemination Level (PU or CO)	Deliverable type (Dropdown Menu)
D6.02	Identification of best practices and guidelines	6	30		UNIMI	PU	Manual
D6.03	Multicriteria analysis results	6	32		UNIMI	PU	Report
D6.04	Internal report	6	32		UNIMI	CO	Report
D6.05	First draft of the User's manual	6	34		UNIMI	PU	Manual
D6.06	User's manual	6	36		UNIMI	PU	Manual
D6.07	Video of presentation of the project	6	36		KOROLEV	PU	Video
D7.01	Press releases	7	2		UNIPI	PU	News Item/Press
D7.02	Communication Plan	7	2		UNIPI	CO	Working Paper
D7.03	Project website & Facebook, Twitter and LinkedIn accounts	7	2		UNIPI	PU	Website
D7.04	Project's corporate identity set, presentation, promotional material, etc.	7	3		UNIPI	PU	Website
D7.05	Plan for the dissemination and exploitation of results	7	7	24	UNIBO	CO	Working Paper
D7.06	Electronic newsletters	7	7		UNIPI	PU	Newsletter
D7.07	Exploitation agreement	7	12	24	UNIPI	CO	Working Paper
D7.08	Joint papers published	7	12		All partners	PU	Journal Article
D7.09	Project video	7	24		KOROLEV	PU	Video
D7.10	2 workshop proceedings and 1 conference proceedings	7	36		All partners	PU	Conference Proceedings

5.1 Tasks

Task 1.1.

The set-up of the SIMTAP prototype at the University of Pisa was delayed by 9 months due to the lack of funds (a results of slow procedure laid down in Italy) and then to the pandemic and the lockdown between March and June 2020, which hampered the construction of the prototype by the company selected at the end of 2019 and restricted the access of the UNIPI staff to laboratories and experimental facilities. This delay has affected the activities foreseen in WP2 (tasks 2.1, 2.2. and 2.3) and may affect WP3 (task 3.1, 3.2 and 3.3) in 2021. During the lockdown, however, it was decided to focus on laboratory scale experiments on polychaetes. Therefore, a laboratory was equipped for these experiments; this laboratory is described in Task 1.1.

The installation of the prototype at MEDFRI campus was delayed due to extension of building by sub-contractor and then the installation was behind the schedule in the pandemic period. Therefore, the adaptation of halophyte plant seedlings and polychaete worms was not realized under high temperature of the summer season. Although fish production in the system is in progress, all components of the system including the sections for halophytes, polychaetes and microalgae could not be run simultaneously. These delays were affected the activities of WP2.

Task 1.4. The lack of funds and the lockdown due to the pandemic strongly delayed all the activities related to the Task 1.4, which is leaded by UNIBO, also as a consequence of the delay occurred to other tasks of WP1 to which the development and test activities of task 1.4 were connected. The delay in material procurement and the restriction of travelling have caused delays in the ISMaCS design, development and tests, for which on-site activities and joint works are fundamental.

Task 2.3. Besides the delays coming from the Task 1.4, the pandemic situation is also directly affecting important on-site activities necessary to carry out the Task 2.3. This has caused a delay in the implementation of the ISMaCS in the SIMTAP installations by UNIBO; consequently, the collection of data needed for energy efficiency analysis of task 2.3 was also delayed. As consequence of the COVID-19 restrictions, industry productions and international expedition are slowed down creating delays to materials order and deliver. For this reason, the provision of specific sensors has facing uncertainties in delivery time. Moreover, the difficulty to travel and move freely among countries makes the ISMaCS implementation in the SIMTAP installations almost impossible, with the exception of the Italian one. The partners are evaluating alternative methods to install the systems that are compatible with the COVID restrictions.

Regarding UNIMI, no funds were given from the Italian Ministry as well as no fund anticipation was granted by the University. Consequently, until now, the expenses for the participation to the kick-off meeting as well as for the technical visits carried out in aquaculture farms (total amount about 5500 €) were anticipated using, temporarily, other funds of the research group.

5.2 Use of resources

There were no important deviations of the use of resources reported in the proposal as regard all the partners. However:

- the Turkish lira has lost value about twice against the Euro in the two years since the project proposal. For this reason, the MEDFRI's budget allocated to the organization of meetings and symposia in the last year of the project has also lost value by half. Therefore, there can be a need for additional funding to participation of the meeting and symposium for the next period.

- UNIMI did not receive any fund from the Italian Ministry as well as no fund anticipation was granted by the University. Consequently, until now, the expenses for the participation to the kick-off meeting in Pisa as well as for the technical visits carried out in aquaculture farms (total amount about 5,500 €) were anticipated using, temporarily, other funds of the research group.

- As regards the implementation of the ISMaCS in the SIMTAP installations (Task 1.4), after a careful analysis of monitoring systems available on market and meetings with partners, the UNIBO team directly built the largest component of the ISMaCS; for the data collection only (server and cloud), commercial solutions were chosen. This ISMaCS design allowed more precise and adaptable systems, better meeting the project needs. This solution entailed to use more resources allocated for "Consumables" and, consequently, just a small part of "Equipment" funds has been spent. Moreover, the COVID-19 restrictions reduced the possibility to participate to conferences, therefore the funds for "Travel and subsistence" and "Other costs" were reduced. Finally, the reduction of "Equipment" and "Other costs" funds, balance the extra costs needed for "Consumables". Despite these transfers in cost categories, the total of UNIBO budget does not require any adjustment as visible in Table 1.

5.2.1 Unforeseen subcontracting (if applicable)

Not applicable for all the partners

5.2.2 Unforeseen use of in-kind contribution from third party against payment or free of charges (if applicable)

UNIPI

The costs for the realization of the DFFO laboratory (see WP1, Task 1.1) and to purchase some instruments and consumables necessary for the construction and management of the SIMTAP system were covered by other funds in the availability of the project coordinator. A research assistant (with MSc degree), Dr. Lorenzo Rossi, was also appointed between November 2019 and October 2020 and the cost of the contract (namely, "borsa di ricerca" in Italian) was covered by the Coordinator's funds. Overall, these expenses were 19,237 €. Since November 2020, Dr. Lorenzo Rossi is a Ph.D. student at the University of Pisa and his project includes many activities conducted by UNIPI in the SIMTAP project. Moreover, a research fellow (post-doc) from Mexico, with a grant from CONACyT (Consejo Nacional de Ciencia y Tecnología Stato di San Paolo, Messico), was hosted between November 2019 and October 2020 at the University of Pisa to work on the SIMTAP project. Juveniles of Gilthead Seabream (*Sparus aurata*) were kindly donated by Maricoltura (INVE Aquaculture Research Centre; https://www.inveaquaculture.com), Rosignano Marittimo (Livorno, Italy). An agreement was signed in 2020 between UNIPI and the Blue Resolution association. ARBI, an Italian private company in the seafood sector headquartered in Monsummano, Pistoia, and the Institutes of Biorobotics and Management of the Scuola Superiore Sant'Anna, Pisa (it is one of three universities in Pisa), belong to this association, which aims to identify and propose innovative solutions for defending the marine environment from pollution. In 2020, ARBI provided frozen seafood, as in-kind contribution (free of charge), for the experiments on fish feeding. UNIPI will inform ARBI about the achievements of the project and will join dissemination activities organized by ARBI on the sustainability of fishery sector.

MEDRI

Juveniles of European sea bass (*Dicentrarachus labrax*) and Gilthead Sea bream (*Sparus aurata*) to study of evaluation of nutritional value of polychaete meal were supplied by Kılıç Deniz A.Ş. (https://www.kilicdeniz.com.tr/). Some of the polychaetes for the pre-experiments was donated by Körfez Av-Yem Dünyası (https://www.yemdunyasi.com/default.aspx) while some was supplied by a local supplier, covered by the Coordinator's funds.

List of attachments

Internal reports

- UNIPI: Internal report: Review on nutritional requirements of marine fish and biochemical composition of detritivores and filter-feeders organisms for SIMTAP systems.
- MEDRI: Report on system starting and biofilter conditioning.

Tools

- DEXi templates and guidelines.

Publications

- Bacenetti et al. (2019). *Approaches to tackle emerging challenges in European aquaculture*. 16th International Conference on Environmental Science & Technology, 4-7 September 2019, Rhodes, Greece. Reprint.
- Barbaresi et al. (2020). *A Smart Monitoring System for Self-sufficient Integrated Multi-Trophic AquaPonic*. Proceedings of IEEE MetroAgriFor 2020 Workshop. Reprint.
- Ciurli et al. (2021). *Multidisciplinary integrated characterization of a native Chlorella-like microalgal strain isolated from a municipal landfill leachate*. Algal Research (accepted). Post-print manuscript.
- Rossi L. Bibbiani C., Fierro-Sañudo J.F., Chinoilema Maibam C., Incrocci L., Pardossi A., Fronte B. (2021). *Selection of marine fish for Integrated Multi-Trophic Aquaponic production in the Mediterranean area, using DEXi multi-criteria analysis as innovative approach.* Aquaculture, 2021, 736402. https://doi.org/10.1016/j.aquaculture.2021.736402.

Dissemination material

- Presentation of SIMTAP to LML students.