Deliverable 6.6: User's manual (PU, 48)

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1 Introduction

Recirculating Aquaculture Systems (RAS) have been emerged as result of the legislative restrictions derived from environmental concerns for conventional aquaculture (Badiola et al., 2012). The use of RAS at a commercial scale has increased markedly during the past two decades (Espinal and Matulić, 2019). Compared to flow-through systems, indoor RASs are closed-loop, land-based systems where the effluent water is processed and reused in production tanks to ensure suitable water quality for maximising growth and fish health under controlled environmental conditions (**Fig. 1**; VFA, 2008; Timmons and Ebeling, 2010).

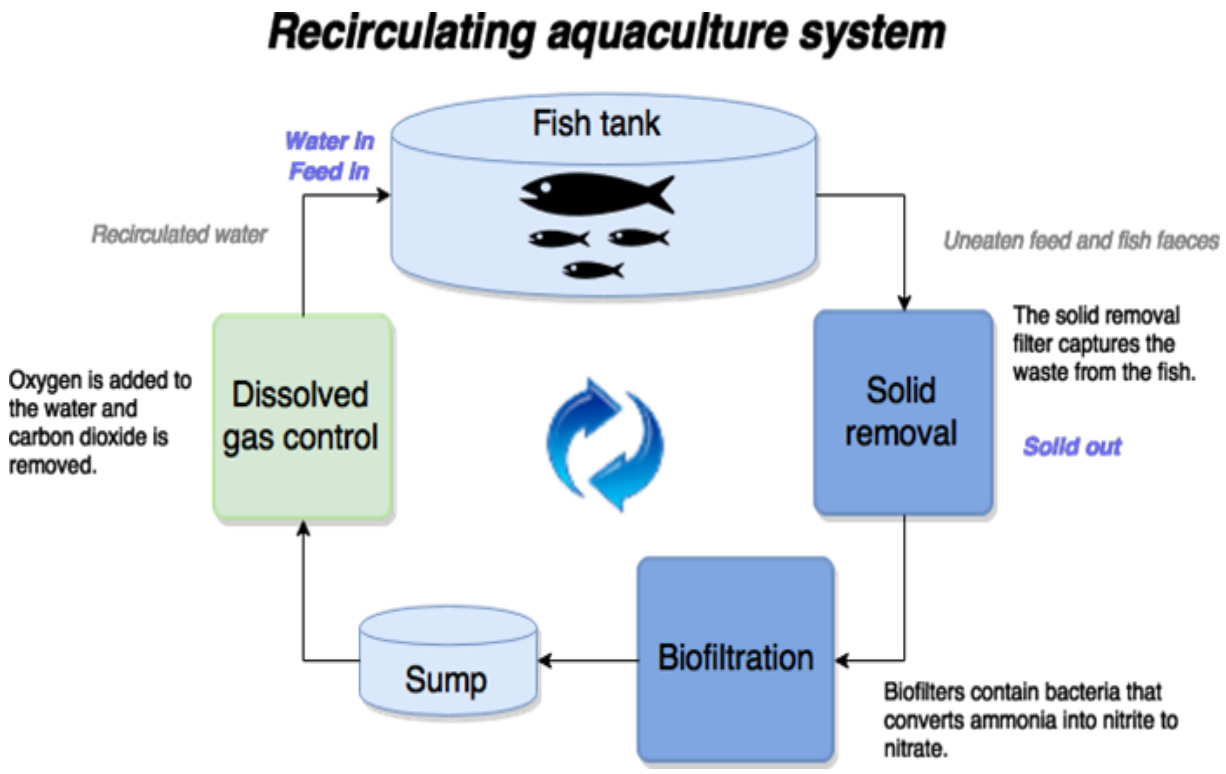


Fig. 1. Schematic overview of a recirculating aquaculture system (RAS).

Recirculating Aquaculture Systems offer several significant advantages over other existing aquaculture systems such as higher profitability with a higher production capacity in a unit area and water use with lower transport costs due to possibility of year-round production in locations close to the main markets (EUMOFA, 2020). These systems can overcome major environmental and social concerns related to conventional aquaculture methods especially including water consumption, solids/nutrients/chemicals discharges, sea space and land use, the transmission of pathogens to the farms and nature, fish escapees, and visual impacts (Tal et al., 2009; Murray et al., 2014). Indeed, many of the environmental issues related to conventional aquaculture systems are about the water

utilization and waste emission (WFA, 2008). The enclosed nature of RAS highly reduces many unfavourable impacts of aquaculture with a more limited land requirement, significantly increased water use efficiency, and decreased waste discharge (Losordo et al., 1998; Piedrahita, 2003). They use 90-99% less water in less than 1% of land area compared with flow-through systems and cage operations (Ebeling and Timmons, 2012).

On the other hand, a RAS operation relies on high technology, which requires high investment and operation costs (Badiola et al., 2012; EUMOFA, 2020)

1.1 The SIMTAP concept

SIMTAP stands for "Self-sufficient Integrated Multi-Trophic AquaPonic systems for improving food production sustainability and brackish water use and recycling". It is an innovative food production system aimed at drastically reducing, on one side, the required fish feed inputs (e.g., fishmeal, fish oil, soybean, etc.) and the consumption of resources (water, energy), and, on the other side, the production of waste and pollution.

The SIMTAP concept 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 as a biological chain (**Fig. 2**). 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.

Moreover, a SIMTAP system can be coupled with the re-use of the effluents from greenhouse soilless cropping systems, in a cascade effect, acting both as a bioremediation of wastewater (run-off) from greenhouse cultivations, and as a recycling of the nutrients still contained in the same wastewater, thus helping the SIMTAP cycle. Besides, the water source for any SIMTAP system can be either brackish or marine. Another application of the SIMTAP concept can be found in system is composed of some earthen ponds connected according to a cascade principle, in which DFFO are also considered as food product.

1.2 SIMTAP in Italy

The SIMTAP system developed in Italy (at University of Pisa) combines fish and plant production in one simple circulation, and thus the same environment, plus a special section aimed at DFFO. Some choices are necessary to obtain optimal growth for the system as a whole unit. To this regard, decoupled aquaponics seems to be more flexible, and it involves several different recirculating loops for the physical separation of the fish, plant and DFFO in separate subunits. A layout of the SIMTAP

system is shown in **Fig. 3**. The systems developed in Turkey and Malta follow the same logic at different facility sizes.

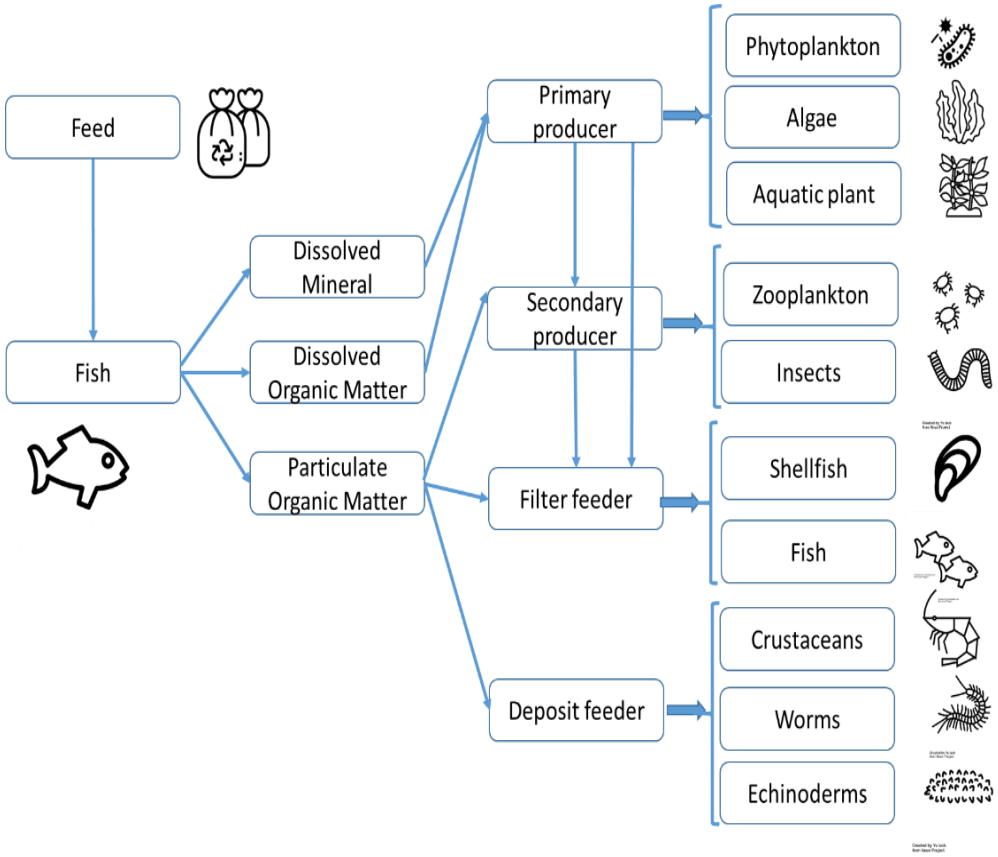


Fig. 2. Biological chain: conceptual scheme.

1.3 SIMTAP in France

The system is composed of five earthen ponds connected according to a cascade principle (**Fig. 4**).

The French SIMTAP is based on three principles:

1. Different species of different trophic levels can be associated in order to reuse the nutrients 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 O2 and CO2 flows. For this objective, a specific model was built based on mass balance and material flow analysis.

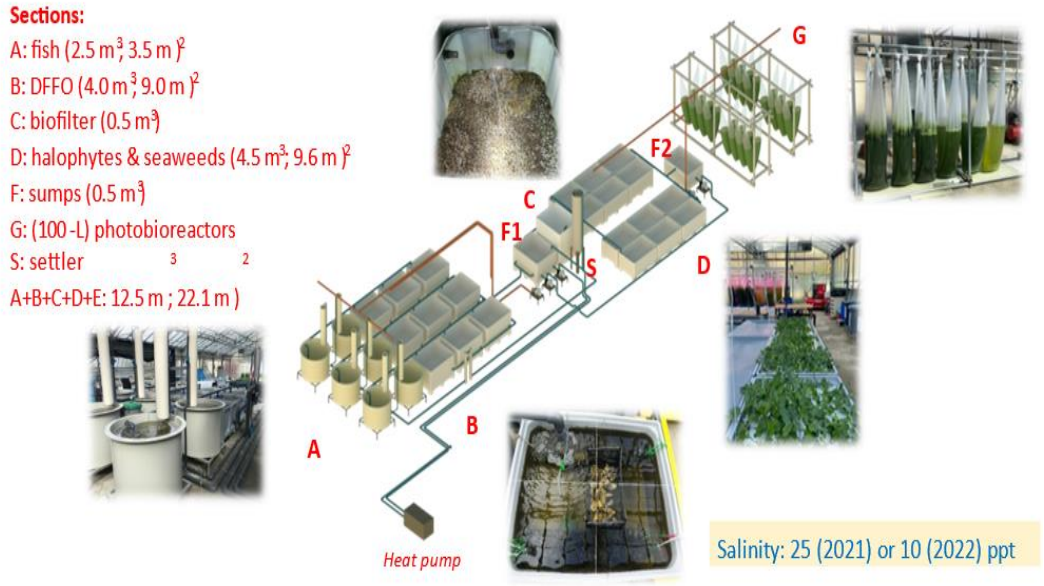


Fig. 3. 3D scheme of SIMTAP at UNIPI. components of the system are listed as: (A) fish rearing tank, (B) FFDO tanks, ($F_{1\text{-}2}$) sump, (S) settler, (C) biofilter, (D) hydroponic and macroalgae section, (G) microalgae section. Pumps, blower, UV steriliser (not labelled) + reversible heat pump (RHP).

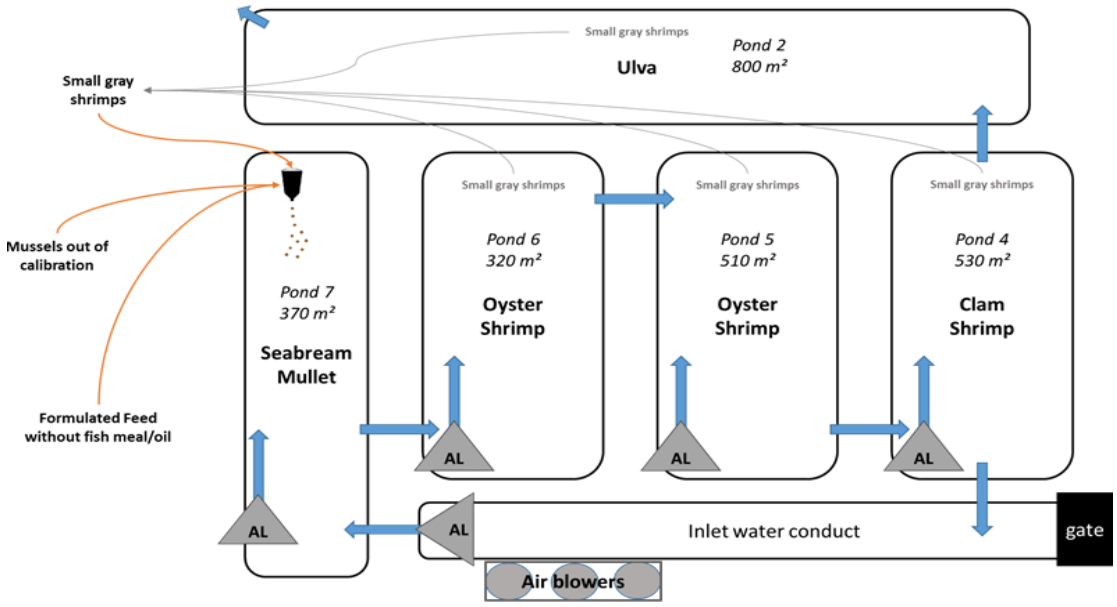


Fig. 4. Diagram of the experimental design of the SIMTAP system at INRAE-LML.

2 Legal issues

2.1 Authorization

In May 2021, the European Commission published a document entitled 'Strategic guidelines for a more sustainable and competitive EU aquaculture for the period 2021 to 2030'. The need to improve the licensing and regulatory framework is a key part of this vision; however, to date, each Member State has had to refer to national regulations for the planning and licensing of aquaculture activities, as there is no centralised international body to manage these processes.

As regards the discharge of wastewater from the system, reference must first be made to directive 2000/60/EC establishing a framework for Community action in the field of water policy and subsequent amendments. The Directive establishes a framework for the protection of inland surface, transitional, coastal, and ground waters, promoting sustainable water use and protection of aquatic environment. This is done, inter alia, through specific measures for the progressive reduction of discharges, emissions and losses of priority substances and the cessation or phasing-out of discharges, emissions, and losses of the priority hazardous substances.

'Basic measures' are the minimum requirements to be complied with and consist mainly of measures required under the following Directives:

- The Bathing Water Directive (76/160/EEC);
- The Birds Directive (79/409/EEC) (1);
- The Drinking Water Directive (80/778/EEC) as amended by Directive (98/83/EC);
- The Major Accidents (Seveso) Directive (96/82/EC) (2);
- The Environmental Impact Assessment Directive (85/337/EEC) (3);
- The Sewage Sludge Directive (86/278/EEC) (4);
- The Urban Waste-water Treatment Directive (91/271/EEC);
- The Plant Protection Products Directive (91/414/EEC);
- The Nitrates Directive (91/676/EEC);
- The Habitats Directive (92/43/EEC) (5);
- The Integrated Pollution Prevention Control Directive (96/61/EC).

These Directives could be integrated by a series of supplementary measures that each Member State within each river basin district may choose to adopt.

2.2 Animal welfare

As far as animal welfare is concerned, basic indications are given in Council Directive 98/58/EC, which lays down the minimum standards for the protection of all farmed animals, and subsequent regulations: Council Regulation (EC) No 1/2005, on welfare during transport, and the Council Regulation (EC) 1099/2009 on welfare at the time of slaughter. However, these regulations make very little direct reference to aquaculture, and have been poorly implemented, in contrast to other farmed species for which specific regulations have been published.

A document called “Guidelines on water quality and handling for the welfare of farmed vertebrate fish” have been published in 2020 by the voluntary own initiative group on fish under the EU Platform on Animal Welfare which was established by the Commission Decision 2017/C 31/12 (Official Journal of the European Union C 31). However, these are intended as a support for the development of future European and national policies and to give supportive indications to stakeholders in the sector but have no legal value. It follows that, at present, any welfare requirements for farmed fish must refer to national legislation. At the national level, animal welfare regulations may also exist in the case of production certification schemes, which may include requirements on husbandry practices (e.g., maximum stocking density), biosecurity standards and more. This is the case, for example, of the production regulations for "Sustainable Aquaculture" in Italy, issued on 4 February 2020 with Ministerial Decree No. 7630.

2.3 Product certification

All products derived from an eventual upscaled and commercialised SIMTAP system, such as the one installed and tested during the project in Italy, Turkey and Malta, cannot be certified as Organic according to the current regulation of the European Union (Reg. 2018/848/CE), which prevents products grown hydroponically from being certified as organic. This is due to the design and structure of the system itself, which is an integrated recirculating multitrophic aquaculture system combined with hydroponics.

Regarding the RAS unit intended to produce fish and macroalgae, at Point 3.1.5. "Housing and husbandry practices" of Part III "Production rules for algae and aquaculture animals" of ANNEX II "Detailed production rules", it is specified that closed recirculation aquaculture facilities and the heating or cooling of artificial water are prohibited, except for hatcheries and nurseries. About the unit intended to produce plants instead, at Point 1.2 of Part I "Plant production rules" of the aforementioned Annex, it is specified that hydroponic production is prohibited. The reason for this is explained in the body of the regulation in Article 28: As organic plant production is based on nourishing the plants primarily through the soil ecosystem, plants should be produced on and in living soil in connection with the subsoil and bedrock.

3 System installation and maintenance

As the SIMTAP system is an experimental prototype, at University of Pisa it was chosen to follow a flexible scheme capable of decoupling the main sections.

The total water volume circulating into the system is around 13 m^3, depending on the pre-set water flow into the fish tanks. That's 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.

3.1 Climate conditioning of the fish section

A heat-pump unit is provided to cope with extreme water temperature that could be faced by the system. The calculation leading to the power requirement for cooling during the summer period showed a minimum Heat Pump power equal to 2 kW. Thus, the chosen an air-to-water reversible heat pump power was 2,5 kW (refrigeration mode).

3.2 Gas stripping

Water aeration in all the tanks and photobioreactors is provided by two blowers, one for the fish and DFFO sectors (living organisms), and the other for the tanks with macroalgae (seaweeds) and halophytes, and the photobioreactors. The blowers can be operated separately.

Before the water runs back to the fish tanks accumulated gases, which are detrimental to the fish, must be removed. This degassing process is carried out by aeration of the water, and the method is often referred to as stripping. The water contains carbon dioxide (CO_2) from the fish respiration and from the bacteria in the biofilter in the highest concentrations, but free nitrogen (N_2) is also present. Accumulation of carbon dioxide and nitrogen gas levels will have detrimental effects on fish welfare and growth. In the trickling system gases are stripped off by physical contact between the water and plastic media stacked in a column.

While in the RAS the water must be filtered to remove solids, ammonia nitrogen and CO_2, in the SIMTAP system at UNIPI, the filtration is also provided by DFFO and, therefore, mechanical filtration should be applied in connection with DFFO unit.

3.3 Mechanical filtration

An operation of RAS requires a continuous water treatment to remove waste products originated by fish feeding and to ensure the optimal water conditions. A basic system consists of solids removal, biological filtration or biofiltration, aeration and degasification (**Fig. 5**).

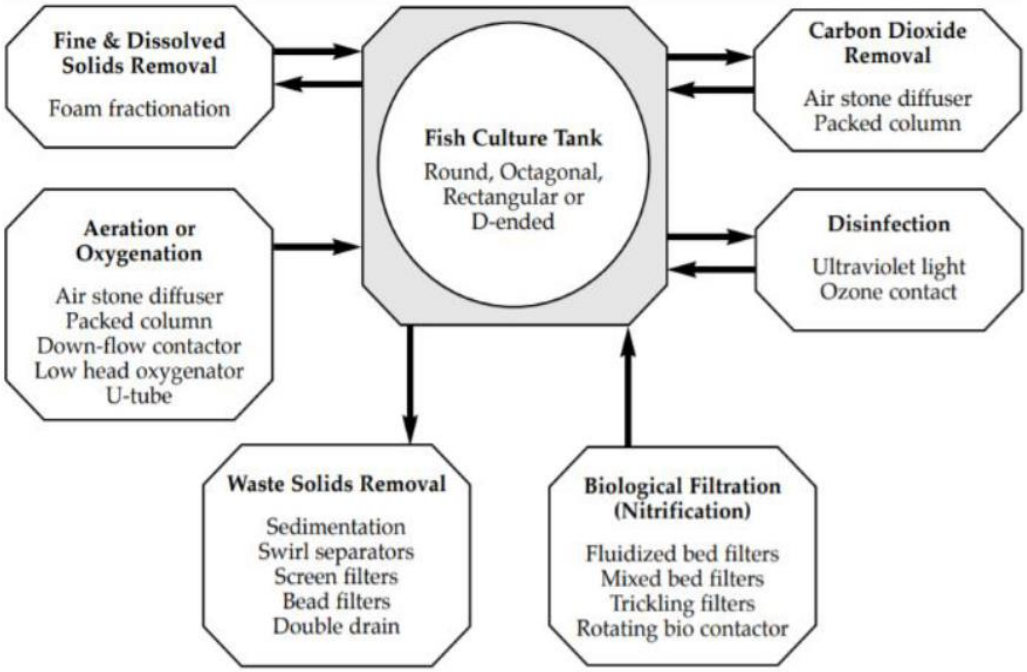


Fig. 5. Some typical components used in recirculating aquaculture systems (Losordo et al., 1998).

Suspended solids negatively impact all aspects of a recirculating aquaculture system (RAS), therefore the first goal of any recirculating treatment scheme is solid waste removal. Suspended solids are the result of faeces, biofloc (live and dead bacteria), and uneaten food. These suspended particles vary greatly in size, from centimetre to micron (μm). The importance of rapid and complete removal of solids from the culture vessel cannot be stressed enough. All other processes on the drive will fail if this primary function is not performed correctly. In RAS systems, most of the particles by weight will be smaller than 100 μm; in intensive RAS systems most particles by weight will be 30 μm or less in size. In these cases, mechanical filtration will be ineffective. From a physical point of view, suspended solids can be further divided into sedimentable solids, generally greater than 100 μm, and non-settleable suspended solids, less than 100 μm. Finer, non-settleable suspended solids are more difficult to control and cause the most problems in recirculating systems. In RAS waters, fine particles (particles smaller than 30 μm) are the most prevalent and dominate the water column. Sedimentation techniques are unable to remove fine particles from water.

There are three methods used to remove suspended solids from farm waters:

1. Separation by gravity. It works on the principle of sedimentation and settling speeds. Unit processes in this category include clarifiers (settling tanks), tubular settlers, and hydrocyclones.
2. Removal by filtration. The removal of particles from water can be achieved with one or more filtration processes. These are sedimentation, filtration, Brownian diffusion, and interception.

These processes are implemented in filtration systems by grid, granular media (GM) or porous media (PM) filters.

3. Flotation process. In a flotation process, particles attach to air bubbles and are separated from the water. The flotation process involves all transport mechanisms that occur in a filtration process, except filtration.

Large particles (above 100 μm) can be effectively removed by tailing ponds or mechanical grid filtration. However, fine particles cannot be effectively removed by either gravity separation or granular filtration methods. Granular filters are only effective in removing particles larger than 20 μm. Microscreen filter apertures range from 40 to 100 μm. Smaller openings remove slightly more total suspended solids and larger openings require less filter surface area and fewer wash cycles. Larger openings require less pressure in the backwash system, and due to fewer wash cycles, trapped waste is often more concentrated in the backwash.

Solid waste removal in recirculating aquaculture is complicated by the fact that poorly aggregated faecal material and uneaten food tend to break down into small pieces in the water. Understanding the role of particle size in the removal process is critical to efficient solids removal.

Relatively large particles, those larger than 100 microns, are considered settleable solids, meaning that if the velocity of the water is reduced, they will sink to the bottom by gravity. Settling or settling is accomplished by allowing the water to slow down, usually in a holding tank, resulting in larger particles falling out. This process can be encouraged by running water through pipes or weirs into the settling tank. The relatively long retention times (slow flow rate) in a settling tank, necessary for settling, are a drawback that has been addressed by diverting some of the flow "offline" (temporarily out of the recirculation loop) so that the settling can take place without affecting the overall flow of the system. Wherever solids settle, they must be periodically removed by draining the sludge with unwanted loss of water. While decanting is cheap and simple, removing particles larger than 100 μm is inadequate: too much solid material is left. Therefore, sedimentation can be used as a primary or preliminary treatment, but not as the sole solids' removal technique.

Adequate solids removal for recirculating aquaculture requires removal of particles up to about 50-75 micron in size. Water with only <50 μm particles remaining will appear somewhat cloudy and turbid but is clean enough for recirculating production aquaculture (but not for display, as in a public aquarium). The most popular removal equipment for smaller particles falls into two categories: granular media and microscreen. A distant third in popularity is the hydrocyclone. The hydrocyclone works by converting the velocity of water into a circular motion in a cone. Solids in the rotating water are thrown to the sides of the cone by centrifugal force. At the sides, the velocity is slower,and the solids sink to the bottom of the cone while cleaner water comes out the top. In essence, a hydrocyclone works by forcing sedimentation into an environment with increased gravitational force (simulated by centrifugal force), not unlike a centrifuge or a merry-go-round. Hydrocyclones are widely used in industry to separate solids from liquids. The disadvantage of recirculating aquaculture hydrocyclones

is that they involve high pressure drop (require additional pumping power), increasing energy expenditure.

Another category of solids removal equipment is porous media. Although they have some special applications, they are not suitable for recirculating production aquaculture. One type of porous media filter is the diatomaceous earth filter. These are mainly siliceous, like sand, and provide a very fine medium for filtering very small solids. They produce clear, sparkling water, but clog very quickly unless the water is pre-treated to displace larger particles.

Another porous media filter is the cartridge filter. A cartridge filter has a replaceable plastic media that traps solids. It has specialized applications in small experimental systems but is rarely used in production aquaculture.

Finally, there is another category of filters that remove the smallest particles (< 30 µm) and colloids. This category includes foam fractionators and ozonisers (ozonation is primarily a disinfection procedure). While it is generally accepted that recirculating aquaculture systems can operate successfully when only particles as small as 50 µm are removed, many operators find it very useful to flush the water with a foam fractionator or ozoniser to minimize problems, maximize production, and reduce the replacement of water that would be used to wash these components.

For the removal of fine solids (< 30 µm), a protein skimmer or foam fractionator relies 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.

3.4 Biofilter

Nitrification is a sequential oxidation of reduced ammonia nitrogen to nitrite and nitrate by microbial processes in a wide variety of environments (Sinha and Annachhatre, 2007). The processes are carried out by three phylogenetically unrelated groups of bacteria: (1) autotrophic ammonia oxidizing bacteria (AOB), (2) autotrophic nitrite oxidizing bacteria (NOB), and (3) heterotrophic nitrifiers (Prosser, 2005). Unless otherwise mentioned, the term ammonia denotes total ammonia nitrogen (TAN; NH_3-N + NH_4^+-N).

Biological filtration is an attached growth process in which a biofilm is generated from the propagation of the bacterial community on an inert surface (called media or carrier), with an advantage of maintaining a higher active fraction of biomass compared to suspended growth in culture water (M'Coy, 1997; Blancheton, 2000). Therefore, biofilters preferred in the RAS operation are based on the fix biofilm on the media named fixed film biofilter which are classified into two main types (emerged and submerged) depending on the strategy used to provide oxygen, and their means of handling biofilm growth (Gutierrez-Wing and Malone, 2006; Malone and Pfeiffer, 2006; Crab et al., 2007). There are several types of biological filters commonly used in RAS with various advantages and disadvantages; trickling biofilters, floating bead filters, fluidized bed biofilters, downflow micro-bead biofilters, and moving bed bioreactors (Pfeiffer and Wills, 2011; Ebeling and Timmons, 2012). On the other hand, the choice of a proper biofilter operated in a RAS is related to

target water quality and treatment efficiency as well as the investment and operation costs (Summerfelt, 2006).

Fixed film biofilters, is a submerged filter presuming that sufficient oxygen can be transported to the biofilm in the water circulated through the filter. This is accomplished using high recirculation rates, internal recycling, or through oxygen enrichment of the influent water. SIMTAP Biofilter is based on MBBR Technology: the biofilm principle with an active biofilm growing on small specially designed plastic carriers that are kept suspended in the reactor, named Floating HDPE Biocell. The carriers are designed to provide a large, protected surface area for the biofilm and optimal conditions for the bacteria culture when the carriers are suspended in water. The recommended random packing biofiltration volume is ½ litre per gram of daily feed (Bioballs® coupled with Deep Water Culture hydroponic section). Typical design values for warm water systems are hydraulic loading rates of 100 to 250 m3/day per m^2; media depth of 0.5 – 1 m; media specific surface area of 300–900 m^2/m^3; and TAN removal rates of 0.1 to 0.9 g/m^2 surface area per day. So, provided a 1000 L bio-filter tank, filled for 1/2 (500 L) with filter biocells of surface area of 450 m2, with a hydraulic load 0.87 L/s/m2, and a target ammonia removal rate by nitrifying bacteria 0.1 g per square meter per day, the calculation of maximum fish biomass results in 120 kg.

Take the example of 20 kg of fish, eating 1 percent of their body weight per day (200 g of fish feed). From these 200 g of feed (45 percent protein), the amount of ammonia produced is approximately 10.0 grams. To achieve this result, first the amount of nitrogen is calculated based on the percentage of protein in the feed; and the amount of nitrogen contained in the protein (16 percent). Then, the amount of wasted nitrogen is calculated: 61 percent of the nitrogen is wasted (6 percent as undigested/uneaten feed retained into the system; 55 percent excreted by fish). For each gram of wasted nitrogen, 1.2 g of ammonia is produced.

The ammonia removal rate by nitrifying bacteria is 0.2–1.0 g per square meter per day. The removal rate depends on the biofilter design, water load (amount of water flowing through the bacteria), temperatures (higher biological activity at > 20 °C), salinity, pH, oxygen as well as suspended solids from fish wastes. To simplify, a conservative rate is used: 0.1 g of ammonia is converted per square meter of surface area per day. Given a daily amount of feed of 200 g and the resulting production of 10.0 g of ammonia, it is necessary to provide bacteria with an operating surface area of 100 m^2. Thus, provided an operating surface area of 450 m^2, the total fish biomass that the biofilter could bear is roughly 4.5x20 = 90 kg.

Anyhow, there is very limited information on the impact of salinity on nitrification. Salinity is similar to both temperature and pH, in that nitrifying bacteria can acclimate to almost any salinity range, given sufficient time. Some authors reported that many engineering companies suggest that the average removal rate is reduced by approximately 37% in salt water compared to fresh water, and that data from commercial fish farms operating at a salinity of 21-24 ppt indicated that the nitrification rate was approximately 60% of what would be expected in a freshwater system.

Because establishment of a nitrifying biofilm under sea water conditions takes a relatively long period (Chen et al., 2006; Rusten et al., 2006), ammonium chloride can be added into recirculating water on 5th day to promote the bacterial growth. While the concentration of ammonia nitrogen was increased to 5 mg N/L, a commercial mixture of selected bacterial cultures (RemoverNH_3, Équo S.r.l., Prato, Italy) was also inoculated into MBBR according to the manufacturer instructions on 5th day from the beginning of test.

3.5 Water disinfection unit

Filtration, heat, UV or ozonation treatments are water treatments that can be applied to contrast the spread of potential pathogens in water, without harmful effects on fish and plant health. Heat treatment is a very effective method against pathogens; however, it needs a lot of energy and also kills the beneficial microorganisms. Filtration also removes plant debris, algae, small particles, and some soil-borne pathogens, and it is the most used technique because of its effectiveness and lower cost compared with the other systems.

Application of chemicals to disinfect the recirculating water is limited. Ozonation (or ozonization) removes all pathogens, including viruses in certain conditions, and ozone is rapidly transformed to oxygen, thus avoiding toxic effects on both fish and plants. However, ozonation can produce by-products and oxidants that must be removed from the water, and oxidizes the mineral elements dissolved in the water, making them unavailable for plants.

The most common methods adopted for water treatment in soilless cultivation and RASs is disinfection by UV (200 to 280 nm), whichdirectly damages the DNA of microorganisms. Continuous activity of the UV lamps and reduced load of suspended solids increase the efficacy of water treatment.

4 System monitoring

4.1 Integrated Smart Monitoring and Control System

The Integrated Smart Monitoring and Control System (ISMaCS) developed during the project is designed to allow the acquisition, storage, and management of large datasets of physical and environmental features in an integrated agriculture and aquaculture context, for the monitoring and analysis of key parameters of the entire production process, allowing assistance in production control, evaluation of system efficiency, and environmental assessment.

*The acquisition system is made up of the following components: sensors, nodes, gateway, and cloud.*Sensors

Sensors are the device used as transducers that convert the physical quantity of interest into an analog or digital electrical signal, which can be acquired by electronic instruments. The ISMaCS can support both commercial-type sensors, with industrial communication interfaces, and custom type.

The Nodes are the components that include a configurable electronic card. They are installable on-site, which can acquire data from the sensors and having processing and transmission capabilities, send via radio signal, on the 868MHz ISM band with LoRa modulation and LoRaWAN protocol, the information of interest to the Gateway. They are designed to be plug-and-play devices

The Nodes work on batteries, charged from various sources, to adapt to any context of use:

- 220VAC electricity grid
- low voltage network 5VDC
- integrated photovoltaic solar panel.

Each Node can be interfaced to a set of sensors to acquire different physical quantities for each point of interest. Even though any sensor can be attached to a Node, and each Node can support more than one sensors (as long as sensor are compatible with the Node interface), the ISMaCS node are showed in **Table 1**.

The Gateway is the device installed in the field and equipped with an Internet connection, which receives the radio information sent by the Nodes and forwards it to the Cloud. The internet connection can be made via a wired ethernet connection (if present on the site) or via a 4G connection. This is the only component installed in the field that requires direct access to the Internet and to the energy grid (or other 220VAC source).

The Cloud component is the set of software applications, allocated on remote servers, which receive the information coming from the field, through the Gateways, manage it, save it permanently and securely on the Database and make it accessible to users through easy-to-use graphs. consultation or for offline download. The access and download are protected with username and password.

Table 1. ISMaCS node and corresponding parameters measured

Node name	**Measured metrics**
Weather	T, rH, air pressure, wind speed, wind direction, rain, illuminance
Greenhouse	T, rH, air pressure, illuminance, PAR, CO_2
Environment	T, rH, air pressure, illuminance,
Energy	Voltage and power on three lines
Water	DO, pH, EC, T

The gateway must be positioned to facilitate radio communication, if the Nodes are positioned in open field at distances greater than 100m from the gateway this must be installed outdoor, alternatively it is sufficient for the Gateway to be located in the same structure where the Nodes are installed.

The gateway (in ethernet configuration) is supplied with a PoE power supply, to facilitate its wiring and to be able to wire a single cable from the power supply to the Gateway. The PoE power supply

must be connected to the power supply, and the ethernet cable of the local LAN must be connected for internet access. Only one ethernet cable must be wired from the PoE power supply to the Gateway. The default configuration allows the Gateway to acquire the network settings from DHCP and is sufficient to allow the Gateway to automatically connect to the Cloud part and be ready to forward information from the local LoRa network to the remote server. Once the user has got the username and password can access to the cloud anytime anywhere to see real time and download data

The Gateway is equipped with an integrated or external higher dBi antenna to facilitate radio reception, furthermore, being equipped with an IP67 waterproof enclosure, it can also be installed outdoors or in industrial environments characterized by the presence of water or dust.

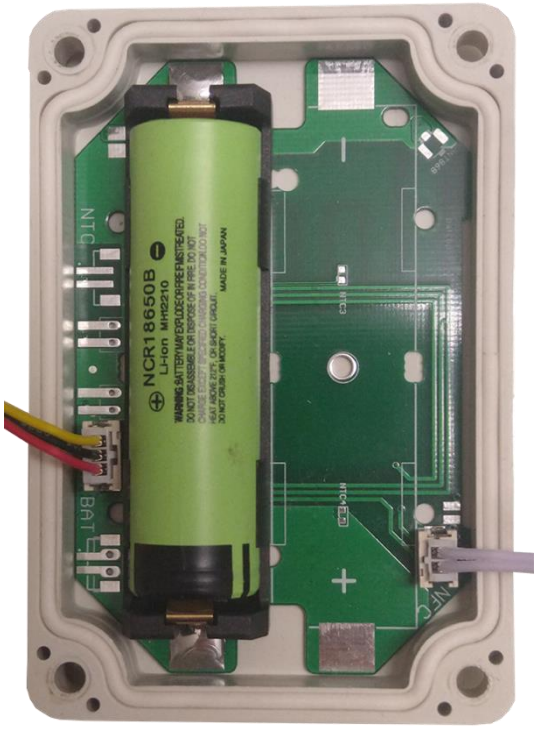
Once the measuring points of a specific application have been chosen, the monitoring system is supplied already pre-configured with the Sensors wired to the respective Nodes. In this way the installation is facilitated, and they will only have to be positioned.

Furthermore, based on the position within the system to be monitored, the single Node is already set up with the appropriate power supply system (**Fig. 6**):

- Power supply from 220VAC grid: The Node is already set up with an internal AC/DC power supply, so that it can be wired to the nearest 220VAC power supply in the system.
- Low voltage power supply 5VDC: In specific application scenarios it is necessary to have many Nodes in small structures. In this case, to optimize the installation, it is useful to set up a low voltage network with a single AC/DC power supply per structure and brings the power supply to 5VDC for all Nodes.
- Power supply with photovoltaic solar panel: to avoid a dedicated power supply for Nodes positioned outside and at a great distance from the main structure, the Node can be equipped with an integrated photovoltaic panel, which guarantees power to the Node in the long term, making it energetically autonomous.

In all the settings, the battery is necessary and ensure the nodes to work even when the main energy supply is interrupted. The battery can last from a few hours up to several days, according to the energy required by the sensors connected to the Node. Some examples of nodes and related sensors installed in the monitoring systems for the SIMTAP project are reported in the next page (**Fig. 7**)

The Weather Node is already supplied with an integrated photovoltaic solar panel which allows an autonomous power supply (**Fig. 8**). The Node has the following integrated sensors: T/H, Lux, Atmospheric pressure. The Wind Impeller Sensor, Wind Direction and Rain Gauge are supplied already mounted on the pole. Install the Wind Sensor rod, taking care to direct the direction marked on the sensor with the letter "S" towards the South. Use clamps or similar tools to fix the Node on the rod of the Wind Sensors, orienting the node with the integrated solar panel towards the south. Connect the Wind Sensors 4-pin data cable connector to the Node. The node is already enabled and proceeds autonomously to acquire the measurements and transmit them via radio to the Gateway.



Standard battery or 5VDC power supply

230VAC power supply

Powered by Photovoltaic Solar Panel

The Greenhouse Node has the following integrated sensors (**Fig. 9**): T, rH, Lux, Atmospheric pressure, CO_2, PAR. Install the PAR sensor and the relevant support bracket have been installed and the node must be secured to a support using cable ties. The Nodes are already enabled and proceeds autonomously to acquire the measurements and transmit them via radio to the Gateway. Note that the Environment Node do not include CO_2 and PAR.

About the energy node, first, install the supplied Electricity Meter Sensor and Follow the following wiring diagram (attach diagram (**Fig. 10)**. Once the Sensor is connected and powered correctly, connect the Modbus RTU communication cable from the electricity meter to the Node. Secure the Node to a support using cable ties. Connect the Node power supply to the 220V mains using the cable gland connector. The Node is already enabled and proceeds autonomously to acquire the measurements and transmit them via radio to the Gateway.

The Water Node has the following integrated sensors (**Fig. 11**): water temperature, PH, electrical conductivity, dissolved oxygen. The Node comes already pre-wired with all the Sensors. Place the Sensors in the water so that the Probes are completely submerged. (Warning: some probes require that they are always immersed in water otherwise they will get damaged). Secure the Node to a support using cable ties. Connect the 5VDC power supply, previously wired in the structure, to the node using the appropriate connector with cable gland. The Node is already enabled and proceeds autonomously to acquire the measurements and transmit them via radio to the Gateway.

Fig. 8. Weather Node.

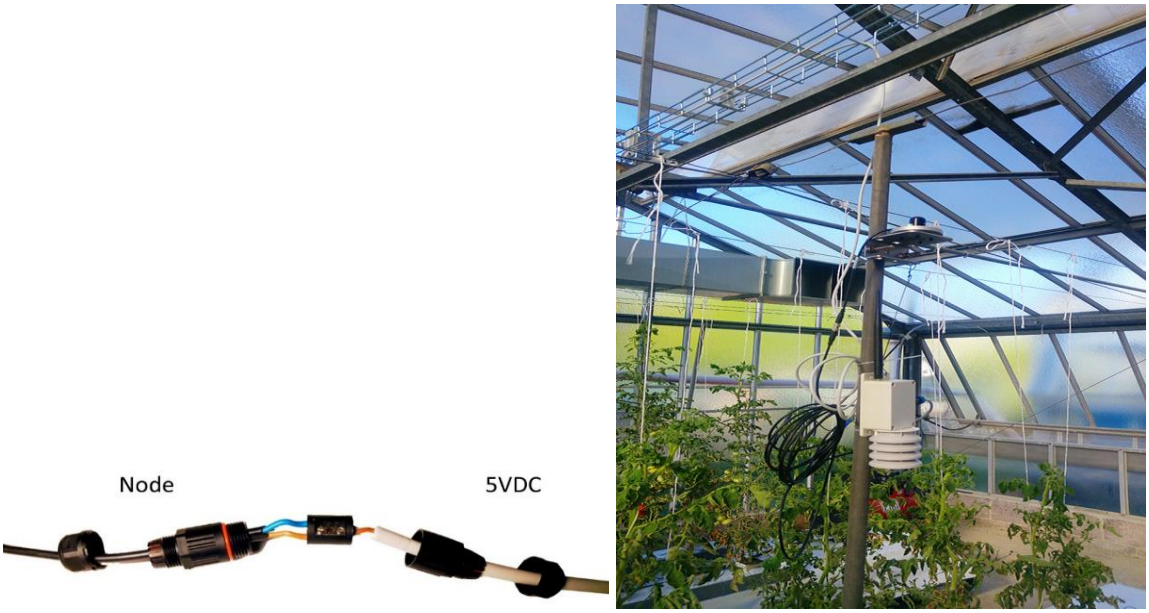


Fig. 9. Greenhouse and Environment Node. Node is supplied ready for 5VDC low voltage power.

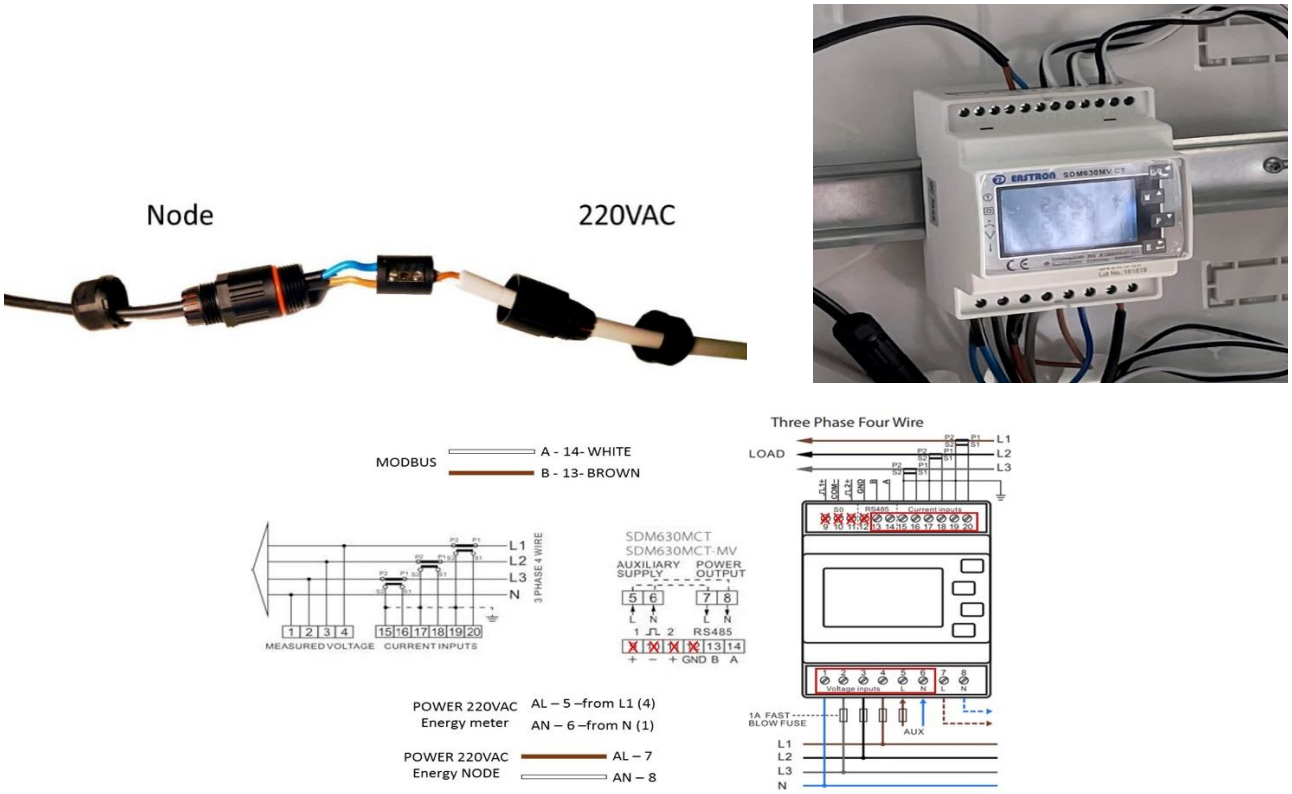


Fig. 10. Elecricity node. The Node is supplied ready for 220VAC mains power supply.

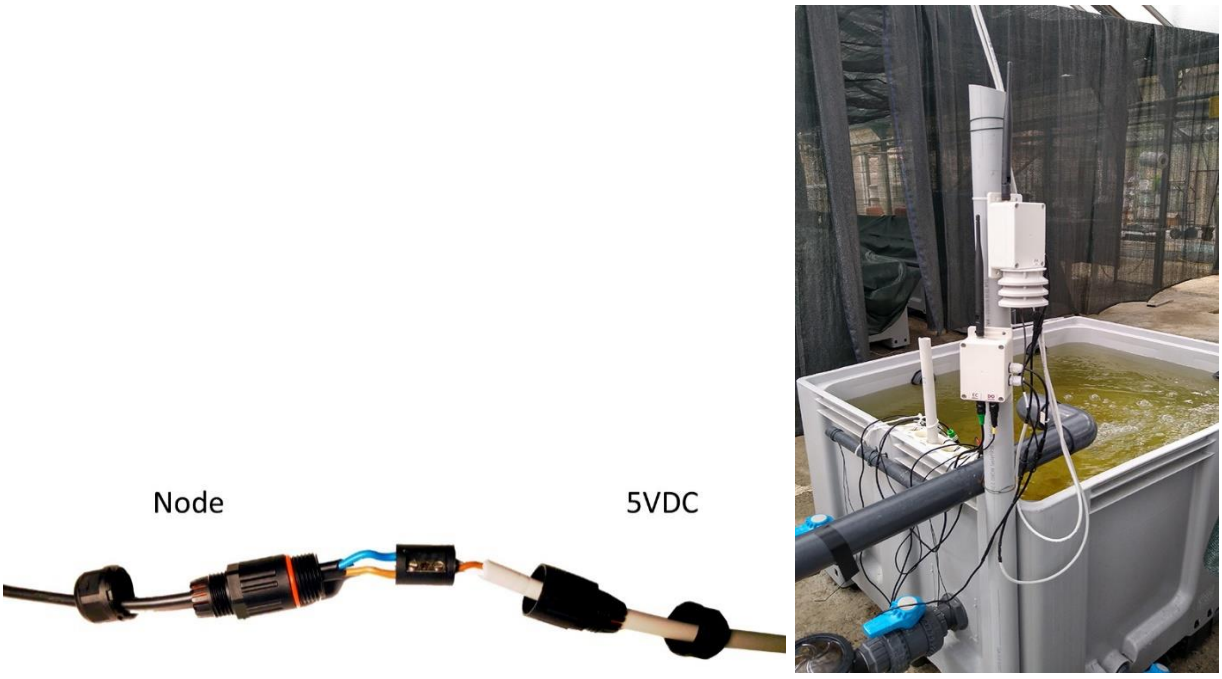


Fig. 11. Water node.

The ISMaCS main architecture do not require any specific maintenance procedure. Please check periodically the data on the cloud. If long failures are noted the system must be revised. On the contrary, sensors need periodical maintenance according to the specific datasheet.

5 Hydroponic cultivation of crop plants

5.1 Introduction

Aquaponics systems are based on two production technologies: hydroponic systems for crop production and RASs for rearing fish and/or other aquatic animal species.

The word “hydroponics” includes all the techniques for cultivating plants without soil in an artificial substrate (aggregate culture) or in aerated nutrient solution (water culture). Since the first application of hydroponics for commercial crop production in the United States more than one century ago, many hydroponic systems have been designed and tested. These systems differ for the presence and the type of substrate and container, the method used to supply the nutrient solution to the plants (drip irrigation or subirrigation, flowing, stagnant or mist water culture), and the fate of the drainage nutrient solution (open system and closed systems). In open systems, the nutrient solution drained out of the substrate at each irrigation event is not reused to irrigate plants. Conversely, in closed systems the drainage nutrient solution is collected and reused into the system.

Almost all plant species can be grown in soilless systems, which however are commonly applied to the production of vegetables, herbs, and cut flowers under greenhouse. Substrate growing systems are used for fruit vegetables (e.g., tomato, pepper, eggplant, cucumber, etc.), strawberry, and cut flowers (e.gl, rose, gerbera, etc.), while leafy vegetables (lettuce, spinach, leafy beet, etc.) and herbs (e.g., basil) are generally grown in water culture (in particular, in floating raft system)**.** The main components of a closed-loop hydroponic system are: 1) fertigation unit for automatic preparation of the nutrient solution; 2) electronic controller interfaced to weather station for irrigation scheduling; 3) water filtration and disinfection unit; 4) monitoring system of the nutrient solution (pH, electrical conductivity (EC), dissolved oxygen (DO), and ion composition).

In an aquaponic system, the ammonium excreted by the fish is converted into nitrate, which is much less toxic to fish, through microbial nitrification. Nitrate tends to accumulate in the water naturally, but its concentration remains relatively low due to the plant uptake. Compared to a hydroponic system, the largest part of the nutrients (>50 %) in aquaponics is provided by fish feed through fish and microbial metabolism. Therefore, microorganisms play a key role in aquaponics, increasing nutrients through mineralization and nitrification and creating a more competitive and resilient environment against pathogens. The presence of fish and plants in the same water loop requires some compromise as regards the characteristics of the water environment and the strategies adopted for fish and crop protection.

Several classifications of aquaponic systems have been proposed, however the most important one refers to the water loop and divides aquaponic installations into two groups: coupled or decoupled (or on-demand) systems.

Coupled aquaponics define a system in which fish and plants are raised in the same water loop. This type of aquaponics is the most studied since many years. In the crop compartment of aquaponic systems, plants are normally cultivated using the following methods: substrate culture with drip irrigation o subirrigation (ebb-flow system or media bed), deep water culture, floating raft system, and nutrient film technique (NFT).

5.2 Plant nutrition

In aquaponic systems nutrients mainly comes from the fish feed and the water added into the system. Fish feed formulations are generally composed of six to eight ingredients, with the aim of providing fish with a well-balanced diet in terms of proteins, carbohydrates, fats, vitamins, and minerals. Protein content ranges from approximately 25% for herbivorous or omnivorous fish, to up to 55% for carnivorous species in early life stages (Boyd, 2015). Organic carbon ranges between 40-45% of fish food while organic phosphorous is around 1.2% (Eck et al., 2019; Timmons et al., 2018). Approximately 95% of feed is digested by fish and only 30-40% of this share is converted into new biomass; the remaining 60-70% is released by fish (gill excretion, urine, and faeces), of which ammonia is one of the major constituents. The last 5% is represented by uneaten feed. Ammonia is produced as end-product of protein catabolism and is consequently excreted by the fish trough the

gills as un-ionized ammonia (Timmons et al., 2018). Ammonia exists in two forms in water: ionized (NH_4^+) and un-ionized (NH_3). The latter is more toxic for fish (Timmons et al., 2018). The sum of both ammonia forms is called total ammonia nitrogen (TAN).

A simple model can be applied to estimate the TAN generated by the fish catabolism of proteins (Wongkiew et al., 2017):

$$TAN = FR \times PC \times 0.092 \quad \text{Eq. 1}$$

where TAN is expressed in g day^{-1}, FR is the feed rate (g day^{-1}), and PC is the protein content of feed (decimal value). This simple equation assumes that: uneaten feed, fish faeces, and sludge are quickly eliminated from the system; proteins contain 16% N; 80% N is assimilated by fish; 80% of assimilated N is excreted as TAN (90%) and urea (10%) (Ebeling et al., 2006).

Ammonium (NH_4^+) is converted into nitrate by a two-step process called aerobic nitrification. In biofilters, ammonia-oxidizer bacteria oxidize ammonia to nitrite (NO_2^-), which in turn is transformed in nitrate (NO_3^-) by nitrite oxidizer bacteria. Nitrification is pivotal to ensure fish health, since ammonia and nitrite are toxic at very low concentrations. Safe values for TAN are below 1 and 3 mg L^{-1} for warm- and cold- water fish, respectively, while the desirable range for nitrite is 0-1 mg L^{-1}. Nitrate is less harmful and represents the major source of nitrogen for plant growth.

Solid wastes are source of phosphorous, potassium, calcium, magnesium, and micronutrients (i.e., iron, zinc, copper, manganese, and molybdenum), which availability to the plants is strictly related to fish feed composition and to microbial solubilisation. Solid waste (faeces and feed leftovers) requires microbial solubilisation (mineralization) to be converted from organic material to the respective ionic mineral forms, which are assimilated by plants. Several other organisms are also involved in the mineralization process, such as worms, nematodes, protozoa, and fungi (Pantanella, 2018). Fish and plants nutritional requirements are satisfied when the ratio of fish feed to plant nutrient uptake is balanced and hence no nutrient accumulation or depletion occurs. Ammonia generation is linked to fish feeding rate and feed protein content, while root nitrate uptake depends on its concentration, environmental conditions, and crop developmental stage (Wongkiew et al., 2017). The content of iron and other micronutrients may be below the sufficiency range for optimal plant growth.

The dimensioning of coupled aquaponic system should avoid nitrate accumulation in water and water discharge balancing the input and output of nitrogen in the system. One of the most common criteria considered is the feed-to-plant ratio, reported as a mass value of feed per unit of plant growing area. This ratio represents the real link between fish (that excrete nutrients through feed consumption) and plants (that use the newly generated nutrients to grow) in an aquaponic system. Thus, the amount of fish feed administrated daily is directly related to the number of plants in the crop compartment. Based on projects' experiences, FAO delivered rule-of-thumb feed to plant ratios: 40–50 g/m^2 per day for leaf vegetables and 50–80 g/m^2 per day for fruiting vegetables (Somerville et al., 2014). **Table 2** summarizes the feed-to plant ratio reported in the literature.

Online open-access calculators exist to determine the optimal area of crop section in aquaponic systems. For instance, the spreadsheet *Aquaponic Media Bed Sizing Model (Ver. 2.0)* is based on

several important inputs (e.g., fish tank volume, fish density, protein content, etc.,) and it calculates the surface area of the media bed that should operate as a biofilter, as a solids mineralization filter, as a plant growing component, or a combination of all three (Lennard, 2012a). The calculator proposed by Lennard, (2012a) is based on a dynamic feed-to-plant ratio adapted to the protein content of the fish diet, starting from a standard value of 60 g/m^2. However, this ratio is strongly affected by several parameters, such as feed quantity, feed protein content, fish and crop species combination, environmental conditions, and system design. Thus, trying to generalize the feed or fish-to plant ratio fails to acknowledge the variability that inevitably occurs in aquaponic systems (Chu and Brown, 2021; Pantanella, 2018).

Table 2. Feed-to-plant ratio in aquaponic system reported in the literature (Pantanella, 2018, adapted).

Crop	Feed-to-plant ratio (g/m^2)	Crude protein content (%)	Fish species	Reference
Leafy vegetables	40-50	32	Not specified	Somerville et al., 2014
	60	32	Tilapia	Lennard, 2012a
Fruiting vegetables	50-80	32	Not specified	Somerville et al., 2014
	100	32	Tilapia	Lennard, 2012b
Lettuce	56	32	Tilapia	Rakocy et al., 1997
	16-13	32	Tilapia	Lennard, 2012a
Sweet basil	81-100	32	Tilapia	Rakocy et al., 2004
Water Spinach	15-42	32	African catfish	Endut et al., 2010

5.3 Decoupled aquaponics

In decoupled aquaponic systems, aquaculture and hydroponic units are separate, thus providing a better control of the water environment in both systems. These systems can overcome the problems originated by an unbalance between fish production of nutrients and their uptake by the plants, as fish effluents can be supplemented with fertilisers to satisfy plants demand. Moreover, it is possible to adjust the pH to make an optimal environment for plants. Before being introduced in the hydroponic section, water must be filtered to remove solids avoid clogging of pipelines and drippers.

The main advantages of on-demand coupled aquaponics systems are higher use efficiency of both water and nutrients and higher yields compared to stand-alone hydroponics systems.

On-demand coupled systems are more complex than permanently coupled ones; thus, simple rules of thumbs may not be enough accurate for a proper dimensioning. An integrated multi-loop aquaponics and greenhouse model has been developed based on existing models for both systems (Goddek and Körner, 2019). The model was tested in three different climatic conditions: Faroe Islands, The

Netherlands, and Namibia. Results indicate the importance of crop transpiration dynamics on system and sub-system sizing.

In case of saltwater aquaponics, the higher salinity concentration in the water determines a progressive accumulation of salt in the recirculating water (crops do not uptake Na as the same rate of N or P), thus the salinity of the system is a criterion that should be considered to avoid stress in crops and production losses. To this purpose, a model for multi-trophic saltwater aquaponic system dimensioning has been developed. The model is named SIM^{2}TAP and it can calculate the intercurrent relations among three main sub-systems: RAS, hydroponic SCS, and polychaetes culture. Moreover, a cascade cropping system (CCS) model is included to evaluate the effect of the addition of effluents from hydroponic cultivation into the aquaponic system. The aim of the model is to simulate the mass flows within these sub-systems with a particular emphasis on minerals (i.e., nitrogen, phosphorous, and sodium) and water flows. Based on this simulation a theoretical dimensioning of the three compartments is given to maximize the use of nutrients generated from the highest trophic level of the system (i.e., marine fish).

The model SIM^{2}TAP has been developed using sub-model for each compartment. The tool was developed in Microsoft Excel® (v. 16.73). An overview of the simulator, with the relevant flows highlighted is given in **Fig. 12**.

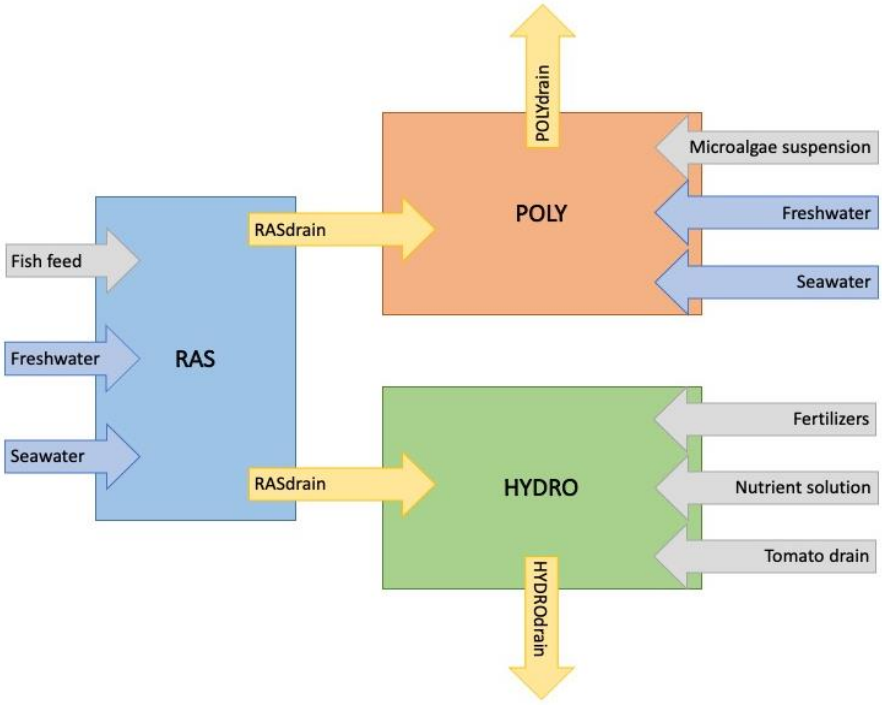


Fig. 12. SIM2TAP model diagram and mass flows.

In the simulator three main sections or compartments are considered: a recirculating aquaculture system (hereafter RAS) for Gilthead Sea bream (Sparus aurata) production, a hydroponic (hereafter

HYDRO) section for halophytes (*Salicorna europeae*) and salt-tolerant glycophytes (Beta vulgaris ssp. maritima), and a section for polychaetes (hereafter POLY) Nereis diversicolor cultivation.

This simulation attempted to answer the dimensioning issue of saltwater integrated multi trophic aquaculture in which euryhaline fish, halophytes and salt-tolerant glycophytes, and polychaetes are produced. As an example, we show the results of the combination of an intermediate value of all the tested variables:

- the overall year production of the system is 9984 kg of seabream,
- percentage of discharged of the hydroponic unit: 25%
- salinity threshold: 17.5 g/L;
- cultivated surface: 5000 m^2;
- nitrogen threshold: 1.75 mM.

Below are the results obtained under these conditions:

- water from RAS drain: 3715 m^3;
- fresh water needed: 2834 m^3;
- nitrogen from RAS drain: 217 kg;
- nitrogen needed as fertilizers: 81 kg;
- percentage of RAS drain not used: 5.2%
- maximum salinity of discharged nutrient solution: 18.5 g/L;
- maximum N concentration in the discharged nutrient solution: 23 mg/L.

The dimensioning criterion for POLY section is the surface required to consume all the solids generated by RAS that, at the same time, allows to reduce the consumption of water, algae suspension, and the release of N and P in the environment. The best trade-off between biomass produced, resource consumption and nutrient discharged is around 550 m2 of polychaetes production surface, for 10 ton of fish produced. Under this hypothesis, the Feed Self production rate is calculated as 10.4%. The overall year production of the system is 9984 kg of seabream, 64651 kg of crops (of which 52869 salicornia), and 5818 kg of polychaetes. In total the consumption of water is 3991 m^3 and 3104 m^3 of freshwater and seawater, respectively. The HYDRO section consumed most of the freshwater (2399 m^3) while RAS consumed all the seawater. In total 5436 m^3 of water are discharged of which 4230 m^3 from HYDRO and 976 m^3 from POLY, almost all the water discharged from RAS (4695 m^3) is used except 229 m^3 (4.9 % of total RAS drain) that are not used in HYDRO due to excessive N in the nutrient solution.

5.4 Crop species selection

In aquaponic systems, the combination of fish and plants should be compatible with the characteristics of each production unit to balance the fish emission of nutrients that are absorbed by the plants. The choice of the proper fish and crop species to be reared in aquaponics is crucial for the success of aquafarms.

Regarding crops, many plant species can be grown in an aquaponic system. The most common plants grown in aquaponics are basil, kale, lettuce, mint, and watercress. In aquaponic, leafy vegetables tend to grow better; if there are enough fishes in the system, also fruit vegetables can be grown, such as beans, cucumbers, squash, tomatoes, peas, peppers, and strawberries. Although freshwater aquaponics is the most widely described and practiced, there is an increasing interest on saline aquaponics to produce euryhaline or saltwater fish, and halophytes or salt-tolerant glycophytes. Saltwater aquaponics does not differ much from the freshwater aquaponics in terms of system design; however, one must consider that nitrifying bacteria are less efficient and plant mineral uptake is reduced at high salinity (Pantanella, 2018; Spradlin and Saha, 2022). Indeed, the rate of ammonia removal rate is markedly reduced (by approximately 37%) in saltwater RAS compared to freshwater systems (Timmons et al., 2018).

Table 3 summarizes the fish and plant combinations in saltwater aquaponic systems and their respective productions, as reported by some Authors. Several commercially valuable fish species have been tested in saltwater aquaponics, such as sea bass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) and witheleg shrimp (*Litopenaeus vannamei*). Several halophytes, such as *Tripolium pannonicum, Plantago coronopus, Salicornia dolichostachya, Salicornia europaea* and *Crithmum maritimum* well adapt to high salinity conditions. All these plants are cultivated for their leaves rich in nutraceutical compounds (Lombardi et al., 2022); some of them (e.g., *Salicornia* spp.) are gourmet vegetables with a high retail price.

5.5 Manual monitoring of aquaponic water

The manual monitoring system consists of all analytical procedures required to monitor the composition of the recirculating water. These procedures can be applied directly on site (e.g., using portable probes) or in specialized laboratories. In fact, many of the necessary determinations cannot be obtained from automatic monitoring systems (e.g., inorganic nitrogen concentration). Moreover, the automatic monitoring system is usually located in specific section of the system (e.g., recirculating tank, biofilter, etc.) while manual monitoring can be conducted in every part of the system allowing also for a "spatial" monitoring of relevant parameters. Therefore, manual monitoring is essential for correct management of the system and to avoid unexpected phenomena (e.g., reduction of the nitrification rate, increase of ammonia concentration). In addition, the manual monitoring can be used to periodically calibrate the automatic system monitoring (i.e., ISMACs). The species reared in an aquaponic system (fish, microorganisms, and plants) have different requirements in terms of water quality. Permanently coupled aquaponics require a trade-off between requirements of different organisms while on-demand coupled aquaponics allow a better management of these parameters. Fish feeding density, growth rate, food intake and quality are among the main causes of the deterioration of water quality. The concentration of macro- and micro-elements in aquaponic systems are often below the recommended level for hydroponics, thus causing plant nutrient deficiency.

Table 3. Summary of experiments conducted in saltwater aquaponics.

Animal species	**Initial fish density (kg m^{-3})**	**Plant species**	**Hydroponic system**	**Density (# m^{-2})**	**Crop yield (kg m^{-2})**	**Crop cycle (days)**	**Salinity (ppt)**	**Authorship**
Sparus aurata	1.2	*Crithmum maritimum*	MB	200	ns	ns	8-20	Vlahos et al., 2019
Dichentrarcus labrax	ns	*Beta vulgaris* (cicla)	MB	50	ns	65	0-20	Nozzi et al., 2016
Dichentrarcus labrax	1.2	*Tripolium pannonicum*	DWC	39	1.0	35	15-16	Waller et al., 2015
		Plantago coronopus			0.6			
		Salicornia dolichostachya			2.2			
Mugil cephalus	8.1	*Salsola soda*	DWC	144	2.2	35	5	Pantanella, 2012
					2.4		10	
	7.4				3.2	32	10	
					2.2		20	
	7.4				5.1	30	10	
					1.0		30	
Sciaenops ocellatus	2.8 - 4.2	*Sesuvium portulacastrum*	DWC	47	3.1*	261	15	Boxman et al., 2018
		Batis maritima			3.6*			

Animal species	Initial fish density (kg m^{-3})	Plant species	Hydroponic system	Density (# m^{-2})	Crop yield (kg m^{-2})	Crop cycle (days)	Salinity (ppt)	Authorship
Xiphophorus	0.8	*Sesuvium portulacastrum*	MB	ns	0.5-0.7	30	15	Boxman et al., 2017a
		Batis maritima			0.3			
Litopenaeus vannamei	200	*Atriplex hortensis*	DWC	100	0.3-0.4	28	10-20	Chu and Brown, 2020
		Salsola komarovii			0.4-0.6			
		Plantago coronopus			0.6-2.0			
Litopenaeus vannamei	200-500	*Atriplex hortensis*	DWC	100	0.6-1.2	28	15	Chu and Brown, 2021
		Salsola komarovii			0.2-0.4			
		Plantago coronopus			2.0-2.5			
Litopenaeus vannamei	250	*Sarcocornia ambigua*	NFT	100	1.1-1.9	73	33.8-36.5	da Silva et al., 2021
Litopenaeus vannamei	ns	*Ocimum basilicum*	DWC	16	6.7	81	1.7	Fierro-Sañudo et al., 2018

Legend: FR, feeding rate; CP, crude protein; FCR, feed conversion rate; SGR, specific growth rate; MB, media bed; DWC, deep water culture; NFT, nutrient film technique; ns, not specified by the author. *Results are expressed on a dry matter basis.

Dissolved oxygen is one of the most important water parameters in intensive RAS, since it determines the successful rearing of fish and the optimal activity of nitrifying biofilter. In general, the oxygen requirement of a RAS is determined by stocking densities, feed addition, temperature, and fish tolerance to hypoxia However, DO level should be kept close to saturation, as fish are very sensitive to oxygen deficiency, in particular at high temperatures. In land-based aquaculture facilities (including RAS), liquid oxygen is used to keep proper DO concentration in water.

The solubility of oxygen depends on temperature, salinity, and atmospheric pressure, as shown in **Table 4**; it decreases with increasing temperature and salinity. Solids originated by uneaten feed, fish faeces, and microbial biomass, could represent an important issue for aquaculture systems due to their accumulation and consequent oxygen consumption by microbial community. Plant roots also are susceptible to oxygen deficiencies, although they are more tolerant than fish. In hydroponics, DO should not drop below 5 mg L^{-1} or 65% of saturation. Hypoxia markedly inhibits plant growth and increases the incidence of root diseases. The risk of the occurrence of hypoxic conditions in the root zone is greater in deep water culture and bed systems than in NFT and aeroponics, where the roots are directly exposed to atmospheric oxygen. Therefore, in aquaponics an automated monitoring and alarm system connected to a backup electric generator is necessary to avoid the interruption of aeration or oxygenation, which will rapidly cause the fish death. The luminescent DO probes are more appropriate for monitoring DO in aquaponics systems, since they do not require stirring and are more accurate than the traditional membrane sensors (Colt et al., 2022).

Table 4. The solubility of oxygen as function of temperature (°C) and salinity (g L^{-1}) at atmospheric pressure of 1013 mbar.

Temperature (°C)	**Salinity (g L^{-1})**								
	0	**5**	**10**	**15**	**20**	**25**	**30**	**35**	**40**
15	10.1	9.8	9.5	9.2	8.9	8.6	8.4	8.1	7.9
16	9.9	9.6	9.3	9.0	8.7	8.5	8.2	8.0	7.7
17	9.7	9.4	9.1	8.8	8.6	8.3	8.1	7.8	7.6
18	9.5	9.2	8.9	8.7	8.4	8.1	7.9	7.7	7.4
19	9.3	9.0	8.7	8.5	8.2	8.0	7.8	7.5	7.3
20	9.1	8.8	8.6	8.3	8.1	7.8	7.6	7.4	7.2
21	8.9	8.7	8.4	8.2	7.9	7.7	7.5	7.3	7.1
22	8.7	8.5	8.2	8.0	7.8	7.6	7.3	7.1	6.9
23	8.6	8.3	8.1	7.9	7.6	7.4	7.2	7.0	6.8
24	8.4	8.2	7.9	7.7	7.5	7.3	7.1	6.9	6.7
25	8.3	8.0	7.8	7.6	7.4	7.2	7.0	6.8	6.6

In addition to DO, the water environment must be regularly monitored to ensure an optimal water environment for fish, plants, and bacteria in aquaponics (Somerville et al., 2014). Water parameters can be monitored manually or automatically with probe or colorimetric kit (Somerville et al., 2014; Thorarinsdottir, 2015). **Table 5** shows the reference values and the frequency of measurements of several water parameters in freshwater aquaponics. In the **Table 6**, references (not exhaustive) to available commercial sensors or test kits are also given.

Optimal pH ranges between 5.0 and 6.5 in hydroponics and between 7.0 and 8.0 in RAS; in aquaponics systems, pH values of 7.0 – 8.5 are optimal for nitrifying bacteria (Lennard and Goddek, 2019). The pH can be monitored directly on-site by using manual or automatic electronic probes (Yanes et al., 2020). Several properties of the water are affected by pH, such as the ammonia form (i.e., NH_3 and NH_4^+, the latter less toxic for fish) (Timmons et al., 2018) or the availability of nutrients for plants. The molar ratio of NH_3 and NH_4^+ in water is related to pH, temperature, and salinity (Timmons et al., 2018): it increases with pH and temperature, as shown in **Fig. 13**, and decreases with salinity. For instance, at 20 °C and pH of 8, the NH_3:NH_4^+ molar ratio is 0.040 in freshwater (0 g L^{-1}) and 0.031 in seawater (35 g L^{-1}).

Table 5. Optimal range of several water parameters and the frequency and method for their monitoring in aquaponic system, according to several authors (Lennard and Goddek, 2019; Pantanella, 2018; Somerville et al., 2014; Thorarinsdottir, 2015; Yanes et al., 2020).

Parameter	Optimal value	Frequency of monitoring	Monitoring method
pH	6 -8.5	Daily	Automatic/manual[a]
Dissolved oxygen (DO; mg L^{-1})	> 5	Daily	Automatic/manual[a]
T (°C)	18-30	Daily	Automatic/manual[a]
Total ammonia nitrogen (TAN; mg L^{-1})	< 1	Weekly	Manual[b]
Nitrite (mg L^{-1})	< 1	Weekly	Manual[b]
Nitrate (mg L^{-1})	5-150	Weekly	Manual[b]
Alkalinity (mg L^{-1})	> 100	Weekly	Manual[c]
Biological oxygen demand (BOD; (mg L^{-1})	< 20	Monthly	Manual[c]
Carbon dioxide (mg L^{-1})	< 20	Weekly	Automatic/manual[ab]
Electrical conductivity (EC, dS m^{-1})	2-4	Daily	Automatic/manual[a]
Total dissolved solids (TDS, g L^{-1})	< 1	Monthly	Manual[c]

[a] By means of hand-held probe. [b] By colorimetric test kit on-site. [c] Laboratory analysis

Table 6. proposed schedule and methods for recirculating saltwater manual monitoring.

Parameter (what)	**Method (how)**	**Commercial kit/test (how)**	**Note**
Temperature	Probe	Eijkelkamp® sensors	If the environmental condition are different (e.g., different locations of system components), the monitoring should be conducted in each part.
Salinity	Probe/refractometer	Eijkelkamp® sensors	In case of mixing with different water source (i.e., freshwater or seawater) it is recommended to repeat measure during time until constant values (dilution effect)
Electrical conductivity (EC)	Probe	Eijkelkamp® sensors	
pH	Probe	Eijkelkamp® sensors	
Alkalinity	Titration		
Dissolved oxygen	Probe	Eijkelkamp® sensors	Considering a gradient in DO concentration due to feeding activity of fish. Repeat the measurement during the day before and after feed administration.
Ammonia	Spectroscopy	Supleco® method (EPA 350.1, APHA 4500-NH3 F, ISO 7150-1, and DIN 38406-5)	Consider a peak of ammonia release due to digestion. Better if measurement is conducted before feed administration
Nitrite	Spectroscopy	Supleco® method (EPA 354.1, APHA 4500-NO2 - B, and DIN EN 26777)	
Nitrate	Spectroscopy	Supleco® method (Zhang and Fisher, 2006)	
Phosphate	Spectroscopy	Supleco® method (EPA 365.2+3, APHA 4500-P E, and DIN EN ISO 6878)	

Parameter (what)	Method (how)	Commercial kit/test (how)	Note
Na, K, Mg, Ca	Atomic absorption spectroscopy		
Fe, Mn, Zn, Cu	Atomic absorption spectroscopy		
Boron	Spectroscopy	Supleco® method (DIN 38405-17)	
Biochemical oxygen demand			Consider this parameter in water effluents, in case of periodical water drain
Chemical oxygen demand			Consider this parameter in water effluents, in case of periodical water drain
Organic carbon		HACH® method (UNI EN 1484:1999)	Consider this parameter in water effluents, in case of periodical water drain
Organic nitrogen			Consider this parameter in water effluents, in case of periodical water drain
Total suspended solids	Gravimetric		Consider this parameter to monitor filtration system efficiency

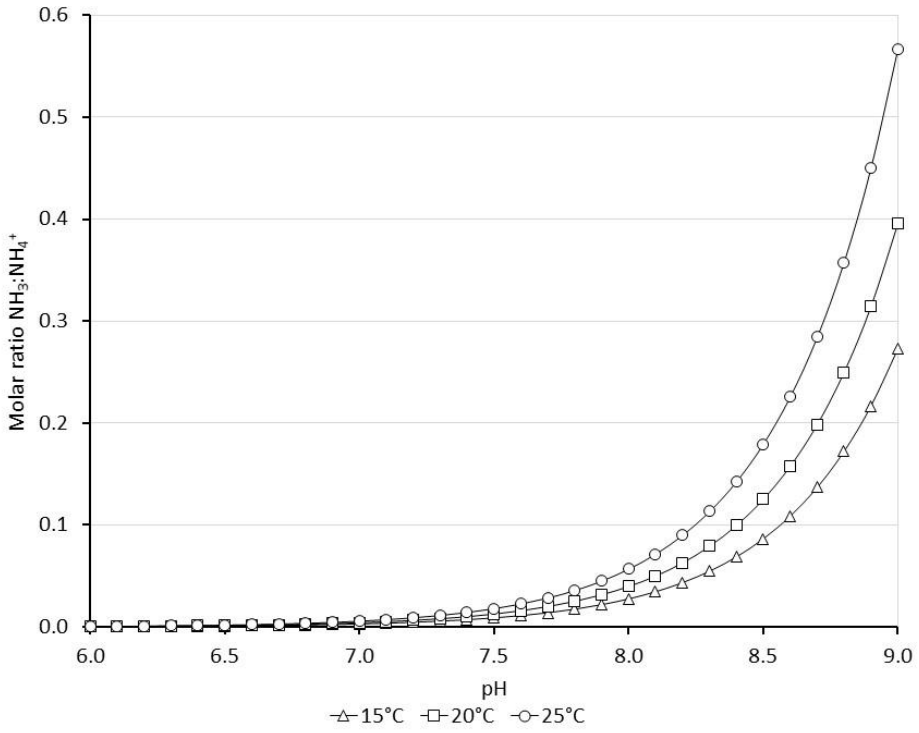


Fig. 13. Molar ratio between un-ionized (NH_3) and ionized (NH4+) form of ammonia in freshwater as function of pH and temperature.

The pH tends to decrease in aquaponic systems and RAS because of nitrification, which favours the presence of ionized ammonia. It is possible to adjust pH through acidification or alkalinization by adding inorganic acids or buffers (salts of calcium, potassium, or magnesium (Goddek et al., 2015; Somerville et al., 2014). The EC is a measurement of the ability of an aqueous solution to conduct electric current, and therefore it is highly correlated to salinity. The EC must be regularly monitored and adjusted to the desired values. Portable potentiometric probes are generally used for EC measurements. In saltwater aquaponics, the salinity can be easily measured with an optical refractometer or a densimeter.

Ammonium, nitrite, nitrate, and phosphorous should be also regularly monitored to avoid toxicity phenomena in fish and nutrient deficiency in plants. These parameters can be quantified in the laboratory with standard analytical methods, which are time-consuming and requires trained staff. or directly on-site using quick colorimetric tests or probes. Specific test kits exist for analysing saltwater in which the presence of chlorine represents the main source of interference for some analytical methods. Other water parameters can be measured less frequently (every 1-2 weeks) such as the total dissolved solids (TDS) and the concentration of micronutrients. The TDS level, which depends on both inorganic salts and organic matter, should be less than 1 g L^{-1} to avoid toxicity for most fish species.

5.6 Crop protection

Root-borne pathogens such as *Fusarium* spp., *Phytophthora* spp., and *Pythium* spp. are the most problematic plant diseases in aquaponics. *Phytophthora* spp. and *Pythium* spp. can spread easily in aquaponics systems because their zoospores easily move in the recirculating water.

In permanently coupled aquaponic systems, the application of pesticides and fungicides is limited by their possible toxicity for fish and beneficial bacteria, and the lack of registered products.Thus, in aquaponics crop protection is primarily based on preventive actions, including water disinfection.

An appropriate identification of the pathogen that cause a specific disease is pivotal to choose the best curative measures. Filtration, heat or UV treatments are physical water treatments that can be applied to contrast the spread of potential pathogens in water, without harmful effects on fish and plant health. *Bacillus* and/or *Pseudomonas* spp. are responsible of the microbial suppressive activity in the filter (Renault et al., 2012) due to the competition for nutrients, antibiosis, and the production of antifungal and/or antibacterial substances.

Heat treatment is a very effective method against plant pathogens; however, it needs a lot of energy and also kills the beneficial microorganisms. Disinfection by UV radiation (200 to 280 nm) directly damages the DNA of microorganisms. Filtration also removes plant debris, algae, small particles, and some soil-borne pathogens, and it is the most used technique because of its effectiveness and lower cost compared with the other systems.

Application of chemicals to disinfect the recirculating water is limited. Ozonation (or ozonization) removes all pathogens, including viruses in certain conditions, and ozone is rapidly transformed to oxygen, thus avoiding toxic effects on both fish and plants. However, ozonation can produce by-products and oxidants that must be removed from the water, and oxidize the mineral elements dissolved in the water, making them unavailable for plants (Gonçalves and Gagnon, 2011).

Other preventive measures can be adopted for the control of crop pests and diseases, such as: use of healthy propagation materials; use of traps and nets; timely removal of infested plants; access restricted to operators with disinfected shoes and clothes; climate control in greenhouse aquaponic systems.

Among the methods to control plant diseases in aquaponics, there are the addition of some microbial metabolites such as biosurfactants (Nielsen et al., 2006), and the use of a complex of antagonistic agents (Vallance et al., 2011)In aquaponic systems, there are beneficial microorganisms that interact with plants and plant pathogens (Sirakov et al., 2016). These microorganisms elicit plant defence response, such as the production of plant metabolites with antibiotic effect and can rapidly develop and spread in aquaponic systems due to the recirculation of water (Vallance et al., 2011).

In addition, the suppressiveness of aquaponic systems depends of the content of organic matter that is contained in uneaten feeds, fish feces, organic substrate, root exudates, and plant residues, and can enhance the presence of antagonistic agents, inducing a microbial ecosystem less favourable for plant pathogens (Vallance et al., 2011; Waechter-Kristensen et al., 1999). On the other hand, a higher C/N ratio increases the abundance of heterotrophic bacteria and reduces that of autotrophic nitrifying bacteria with a negative impact on the system.

6 Seaweeds

6.1 Seaweed management

Macroalgae are interesting alternative/complementary species to crops as inorganic extractive in IMTA. In SIMTAP at the University of Pisa *Ulva* spp. (hereafter Ulva) has been cultivated during 2021 at 25 ppt of salinity for six months. Ulva was cultivated with a continuous supply of water from the seabream (coupled configuration) and the growth and mineral composition was monitored over time. In this section a guide on how to cultivate Ulva in a coupled saltwater aquaponic system is given.

The Ulva could be harvested locally in places with similar environmental conditions (e.g., radiation, temperature, salinity, etc.) to those in which it will be grown later. In our case Ulva was collected in the Orbetello Lagoon site (42°25'56.28"N; 11° 9'39.21"E) in a reservoir of aquaculture wastewater. Transportation can be conducted in tanks or bags filled with local water and under aeration to maintain the algae biomass in suspension. Ulva should be collected during the vegetative phase that start with spring.

Once arrived in the facility, the biomass should be rinsed with clean water, to remove epiphytes, macroinvertebrates, and potentially invasive organisms. Any chemical treatment should be avoided to not damage algae tissues. Ulva is a leafy alga (thalli) and only the best one should be chosen for the introduction in the system (**Fig. 14**).

6.1.1

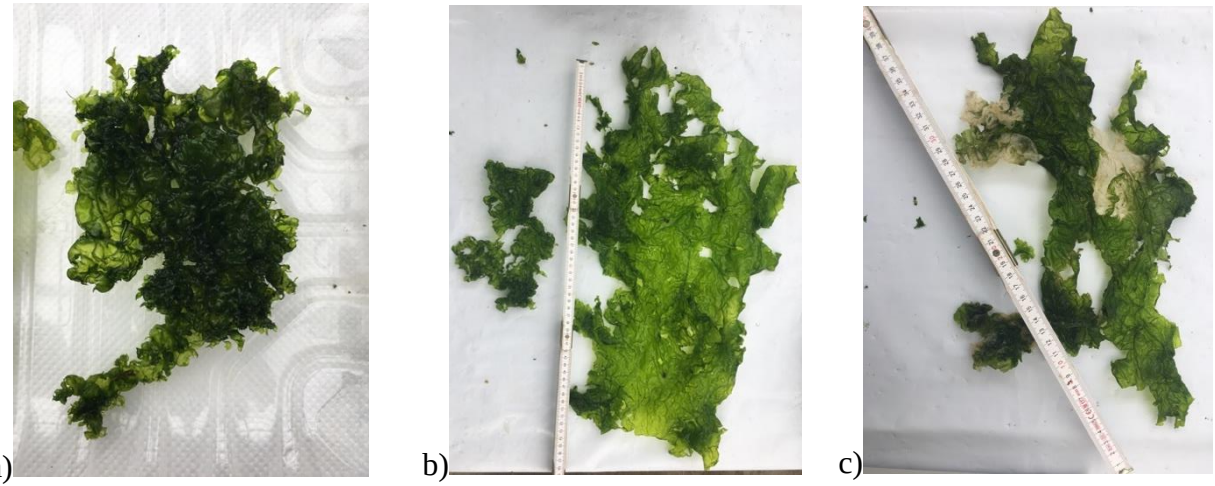


Fig. 14. Ulva cultivated in SIMTAP during 2021. In details, vegetative phase (thalli proliferation) (a), good quality (b), and old/bad quality of Ulva (c) at harvest.

Ulva can be introduced in the system at different biomass densities. In our case, we introduce 1 kg m^{-2} of fresh biomass in 0.8 m^2 rectangular-shaped tanks with 0.3 m of water height. The tank should be cleaned and disinfected before the introduction of the algae. Water should be maintained aerated to avoid stagnation of water and to favor light penetration, especially during intense algae bloom (e.g., air stones/pipes to bottom aerate the water). Alternatively, water can be maintained in movement

by air injection or paddle wheels. To avoid circulation of small thalli or it is recommended to screen the overflow of the tanks with a 0.5 cm net, that should be periodically cleaned (brush) to avoid clogging. A periodical visual inspection is recommended to monitor the growth of the algae that can be easily evinced by the coverage of water surface. Ulva can grow both suspended or attached to the tank walls and bottom. The harvest can be conducted by simply remove the algae from the water with the help of a net. During harvest is better to collect all the suspended form and avoid damaging the attached Ulva. After harvesting algae biomass can be rinsed with freshwater and drained with the help of a net or perforated trays. A part of this biomass (better if the vegetative form, good quality) can be introduced back in the tanks at the same biomass density (i.e., 1 kg m^{-2}).

7 Fish and aquatic organisms

7.1 Species selection

In IMTA systems, fish and other aquatic animals are considered "generative" or "fed" species and usually represent the highest trophic level. The choice of fish species should be based on several criteria referred to physiological, technical, and economic attributes of the animals (**Fig. 15**). These attributes refer to the geographical distribution of the species that can affect consumer acceptance. Moreover, local species are naturally adapted to the rearing environment and the risks related to the introduction of allochthonous species is reduced. For SIMTAP the selection regard species naturally present in the Mediterranean Sea. In addition, the degree of domestication that refer to both the know-how in farming and the availability of juveniles is a fundamental criterion. Species with well-known farming procedures can be easily introduced in SIMTAP systems. The availability of hatchery-produced juveniles, increase the possibility to obtain high-quality fish to be introduced in the system, allow the off-season introduction of juveniles, and reduce at the same time the pressure on wild stocks. The species to be introduced in the SIMTAP should also be adapt to the peculiar farming conditions. In this case, species with tolerance to a wide range of salinities and temperatures, should be preferred. Thus, the choice of euryhaline and eurythermal species should be preferred. In addition, the adaptability to a diversified diet is considered a favourable attribute in the farmed species. According to the SIMTAP approach, fish should be fed diets completely or partly self-produced within the system itself or using locally available ingredients (e.g., French SIMTAP experience, in which the diets were based on ingredients regionally produced and integrated with fresh mussels out of calibration). SIMTAP is designed to produce fish and aquatic animals for human consumption. In this perspective, the growth performances and the quality of the final product should be considered. The profitability of the system is primarily related to the market price of the farmed and cultivated species. Thus, species with high market price as raw products or processed products.

To take into account all the above-mentioned attributes, a multi-criteria analysis was conducted at the beginning of the project. An open-source software (DEXi) was used to create a decision model based on qualitative attributes (DEXi_SIMTAP_Fish_1.0). The DEX approach divides decision-making problem into less complex sub-problems represented by criteria organized hierarchically. The final output of the decision process showed that European sea bass and Gilthead sea bream were the most suitable candidates to be reared within the SIMTAP system.

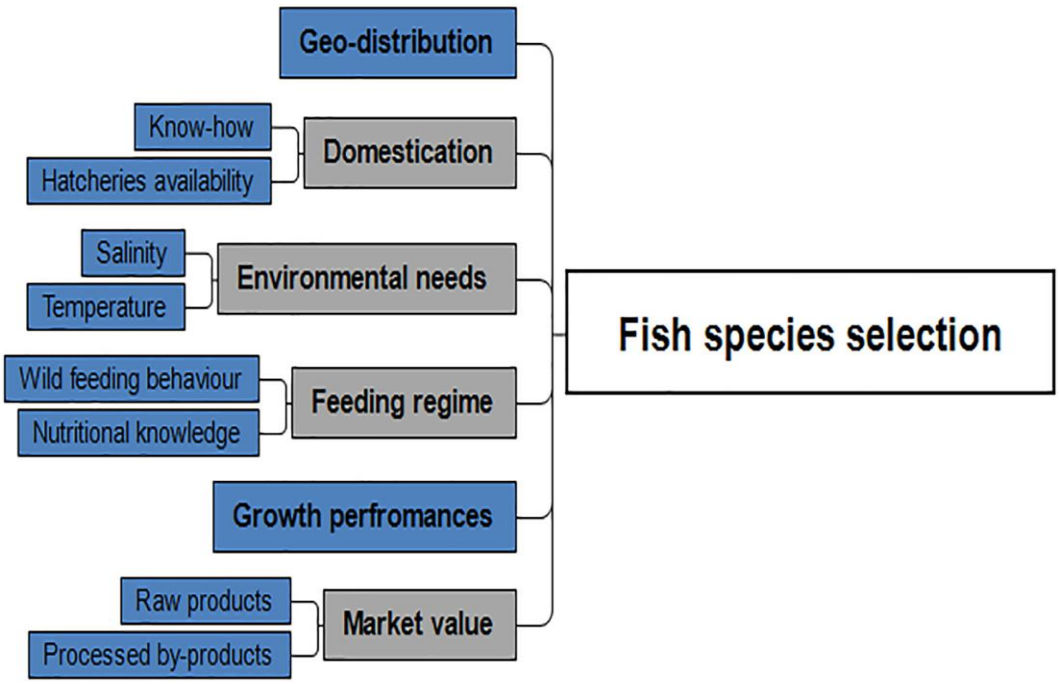


Fig. 15. Example of attributes considered for fish species selection (Rossi et al., 2021)

Strengths of these species were the high domestication level already reached, as well as their market value. These findings do not mean that other candidate species cannot be considered for SIMTAP production or other IMTA systems. In fact, Flat-head grey mullet, Shi drum, Meagre, Turbot or Solea, were demonstrated to be good species for these type of production systems. Worthy to be mentioned is the case of Flat-head grey mullet that represents an interesting species in the perspective of aquaculture diversification and thanks to its fast growth and the interesting market price of its processed products (bottarga).

7.2 System dimensioning

SIMTAP fish production should be scheduled strictly considering several aspects. First, the carrying capacity[1] of the system, that can be defined as the maximum biomass of farmed fish that can be hosted in the system without negatively affecting the proper balance of the whole system (i.e., DFO unit, biofilter, plant production unit, and water-quality parameters). This means that it is absolutely forbidden to reach a fish stocking density that may produce a quantity of wastes that cannot be metabolized by the DFO section (suspended solids), the biofilter (ammonia), and the plants unit (ammonia, nitrites and nitrates uptake), in a medium-long term perspective. Temporary exceeding

[1] "Carrying capacity is defined as the maximum biomass of a farmed species that can be supported without violating the maximum acceptable impacts to the farmed stock and its environment. Maximum acceptable impacts on the farmed stock and the environment are expressed by standards for water quality in the farm and the surrounding environment." (Stigebrandt, 2011)

can be tolerated but the risk of solids and ammonium accumulation and nitrification disruption of the biofilter may occur. For this reason, the system implementation of a suspended solids separator (e.g., sands or drum filter) and the occasional discharge of rearing water can be considered despite they represent a sub-optimal use of SIMTAP system. Based on that, the fish stocking density must closely follow the ammonium plant uptake and suspended solid removal rate of the DFO unit.

To estimate the fish stocking density and its effect on the system carrying capacity, a farm-based model can be built to predict the weight of the fish over time as function of water temperature. Then, based on this information, the consumption of feed and other outcomes (e.g., release of N, oxygen consumption, etc.) can be predicted. Several growth equations for farmed species are already available and they can be specifically calibrated using field-collected data. An example of growth equation and coefficients obtained from fish growth monitoring is given in **Table 7**.

Table 7. Example of equation used to predict fish growth and coefficients obtained from SIMTAP experiments in Italy, for Gilthead sea bream.

Equation	**Coefficients**		
Individual body weight gain		< 230 g	> 230 g
$W_t = \left[W_0^{(1-\beta w)} + (1-\beta w) \times \alpha w \times e^{(\gamma w \times T)} \times t\right]^{\frac{1}{1-\beta w}}$	α_W	0.034	14.922
	β_W	0.864	-0.370
	γ_W	-0.002	-0.003

Equation from Mozes et al., 2011, coefficients through regression from experimental results.

7.3 Stocking of fish

Fish juveniles to be introduced in the SIMTAP system can be purchased either by hatcheries or farms. To this purpose, the stock size, fish price and cost for transportation (e.g., distance) should be considered. The fish stock purchased should also be evaluated for its quality (**Fig. 16**) by sampling a representative number of fish (e.g.,100-200) and observing the incidence of skeletal, fin, and opercular deformities. An acceptable limit of deformities may be 3% (Cardia & Lovatelli, 2015). The stocks should also be checked for pathogen infections (viral, bacterial and parasitic) sending a fish sample to a a specialized fish pathology lab. Moreover, the fish stocks should also be monitored for body weight and lenghts homogeneity. In Gilthead sea bream, a relative standard deviation ($RSD = \sigma/\mu \times 100$; where σ is the standard deviation and μ is the average weight) between 3 and 10% usually can be considered acceptable (Cardia & Lovatelli, 2015).

The fish transportation should be conducted in compliance with local regulations, such as the European Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations, or national/local regulations. Fish should be introduced in clean, disinfected, and well aerated tanks. The environmental parameters of salinity and temperature should be as close as possible to those of the origin. In case of differences in salinity and/or temperature fish should undergo through an acclimation period (and quarantine) for at least 30 days,

while slowly adjusting the water parameters until reaching the final values. Changes in water parameter should be lead gradually (for instance 1 ppt each day, for salinity, 0,5-1,0 °C for temperature) and fish welfare must be daily monitored.

a) b) c)

Fig. 16. Quality control of juveniles at the stocking time (a), sorting (b), and weighing (c) procedures.

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7.4 Growth and periodical monitoring

Also, after the introduction into the SIMTAP system, on a daily base fish must be further monitored by visual inspection. Particular attention must be paid to fish behavioural dysfunctions, damaged or sick fish, appetite, and mortality. This inspection may be lead during feed distribution and water quality manual monitoring.

The initial number and biomass of fish in the system should be recorded as accurately as possible. Data required and equations for growth monitoring of fish is given in **Table 8**.

Table 8. Example of the data and equation for biomass monitoring and parameters estimation in the tanks.

	At the stocking			After the stocking					
Par.	Biomass kg	Average biomass kg	Number of fish	Biomass Sample	Average biomass Sample	Number of fish Tank	Biomass fish Tank	Mortality Tank	Density Tank
	B	BW_a	N	B_s	BW_s	N_t	B_t	M_t	D_t
Eq.	measure	measure	$\frac{B}{BW_a}$	measure	measure	measure	$BW_s \times N$	measure	$\frac{B_t}{V_t}$

Abbreviations: V_t, total volume of the tank

A suitable protocol to be adopted may consists in weighing all the fish biomass and counting the number of individuals. As an alternative, weighing 10% of the individuals can be also a suitable method for biomass and mean body weight estimation, particularly when large stocks are introduced into the system. However, counting the number of individuals introduced in the system must be considered a priority. The growth monitoring should be led periodically by weighing a representative sample of the fish stocked in the system, e.g., 5 or 10% of the stocked fish. Five per cent should be used for large stocks (at least 1 million fish), 10% when the size of the stock is lower. The sampling frequency may vary according to the growth stage of the fish. A suitable compromise maybe monitoring fish body weight each month or each second month for juveniles (up to 100 g body weight) and adults (over 100 g body weight), respectively. By applying this protocol is possible to estimate both the number and the biomass of the fish in each tank and properly calibrate the amount of feed to be supplied daily. Between two different body weight check, the amount of feed supplied must be modified according to the standard growth curve of the considered species. Meantime, the stocking density must be also monitored and reduced in case of need. To this regard, it is suggested maintaining the fish stocking density always below 15 kg/m^3. Fish mortality in each tank should be recorded on a daily base.

According to the data obtained from growth monitoring is possible to calculate the amount of feed to be administrated every day. Feed manufacturers give recommended feeding level for each commercial product, which represent the amount of feed as percentage of the biomass stocked. As showed in **Tab. 9** at constant temperature the amount of feed to be administrated per unit of weight decrease with increasing of fish size. A different approach consists in estimating the feed consumption to satiation by applying existing available models. For seabream, the daily amount of feed to satiation (F) can be estimated according with the following equation and coefficient proposed by (Lupatsch & Kissil, 1998): $F = \alpha_F \times W^{\beta_F} \times e^{(\gamma_F \times T)}$. Where W is the weight of the fish and T water temperature.

The daily feed ration should be distributed in 4-6 meal per day until fish do not reach the body size of 100 g. This enables to promote feed consumption and growth more efficiently. For fish heavier

than 100 g, the number of daily meals can be progressively reduced up to 1 per day without compromising feed consumption and fish growth.

In case of feed with low moisture content (less than 10%) automatic feed dispenser can be used to avoid manual feed administration. Several automatic feed ingredients exists (Fig. 20) and the choice should be made in terms of cost effectiveness (e.g., by taking into account the time required to the administration of feed, the fed wastage, etc.).

Table 9. .Example of recommended feeding level (kg feed 100 kg fish^{-1} day^{-1}) for Gilthead sea bream at different growth stages (https://www.aller-aqua.com/species/warm-saltwater-species/sea-bream).

		Water temperature (°C)								
Fish (g)	MM	12	14	16	18	20	22	24	26	28
20-50	3	0,58	0,82	1,11	1,47	1,96	2,61	3,1	3,27	2,94
50-100	3	0,47	0,65	0,89	1,18	1,57	2,09	2,48	2,61	2,35
100-200	4.5	0,37	0,52	0,7	0,93	1,24	1,65	1,96	2,07	1,86
200-400	4.5	0,3	0,41	0,56	0,74	0,99	1,32	1,57	1,65	1,49
400-600	6	0,24	0,33	0,44	0,59	0,79	1,05	1,24	1,31	1,18

In case of feed with low moisture content (less than 10%) automatic feed dispenser can be used to avoid manual feed administration. Several automatic feed ingredients exists (**Fig. 17**) and the choice should be made in terms of cost effectiveness (e.g., by taking into account the time required to the administration of feed, the fed wastage, etc.).

In case of use of fresh ingredients, the following procedure can be adopted:

- All the ingredients should be periodically analysed for proximate composition: crude protein, crude lipids, crude fibre, ash, and moisture. The gross energy content can be calculated by applying energy yield coefficient (Henken et al., 1986).
- All the calculations should be made in terms of dry matter content because the high moisture content of fresh ingredients.
- The composition of the ingredient mixture (diet) must cover the fish requirements in relation to the fish species and growth stage (NRC, 2011).
- The amount of fresh diet (F) to be supplied can be calculated as follow: $F = \frac{B \times Fr\%}{DM_F\%}$. Where B is the biomass measured or calculated, Fr is the feeding rate in terms of dry matter (alternatively the amount of feed required can be calculated as above illustrated), and DM_F is the percentage of dry matter of the diet.

– The amount of each single ingredient (I) can be calculated as follow: $I = \frac{F \times DM_F\% \times F_I\%}{DM_I\%}$.

Where F_I is the percentage of inclusion of I in the mixture (on a DM basis) and DM_I is the percentage of dry matter of the ingredient I.

Due to their perishable attitude, fresh ingredient should be prepared at least once per day, immediately before the first administration. The ingredients should be combined according to the previously calculated amount, and it is strongly recommended to keep refrigerated portion that will be supplied in the following meals. In case of early growth stage fish, chopping the mixture may be helpful to reduce the fish competition but it must be considered a higher feed biomass loss and nutrient leaching. For this reason, it is recommended to not mince excessively the mixture (**Fig. 18**).

Fig. 17. Automatic feed dispenser available for land-based aquaculture systems. From the left: low-tech belt feeder dispenser (a), automatic disc dispenser for small surface (tank) application (b), automatic blower dispenser for large surface (ponds, basins, raceways) application (c), high-tech automatic dispenser for small surface application with remote control. (Source a, b, c: https://scubla.it; source d: http://www.mirafeedsystem.com/)

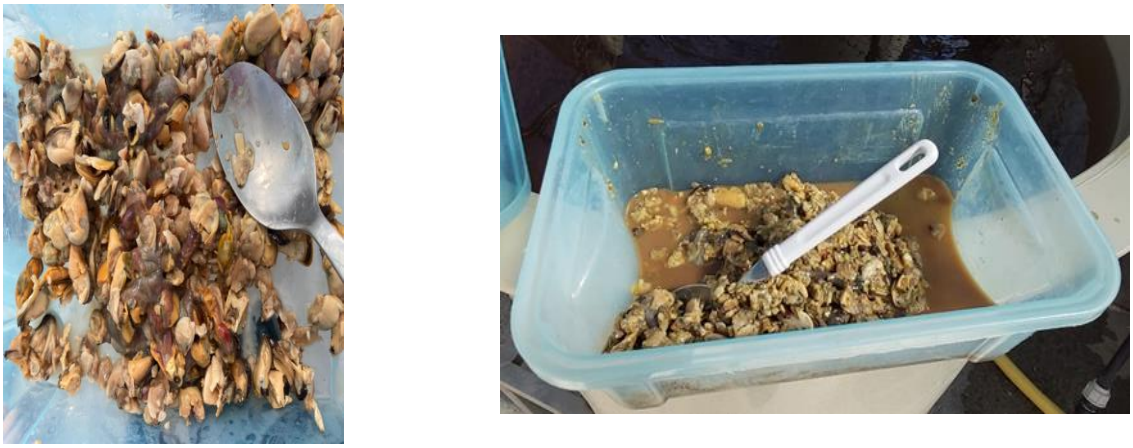

Fig. 18. Correct mincing and administration of fresh ingredients mixture (left), inappropriate administration due to the excess of water in the mixture (right)

8 Intermediate organisms

8.1 Polychaetes

The polychaete *Nereis diversicolor* can be produced in SIMTAP. The production cycle can be divided into two main stages: 1) broodstock maintenance and reproduction and 2) on-growing stage.

Adult worms can be wild-collected and maintained in a separate recirculating system until maturation (hereafter nursery). Then, sub-adults are transferred into the SIMTAP until reaching target size for harvesting for fish feeding. A schematic representation of the process is given in **Fig. 19**. *N. diversicolor* is a semelparous specie, thus, adults die after spawning. This physiological characteristic necessarily requires the adaptation of the rearing protocol and in particular: 1) by favouring the adults maturation and synchronous spawning during the nursery stage and 2) by stimulating growth avoiding at the same time uncontrolled reproduction during the on-growing stage.

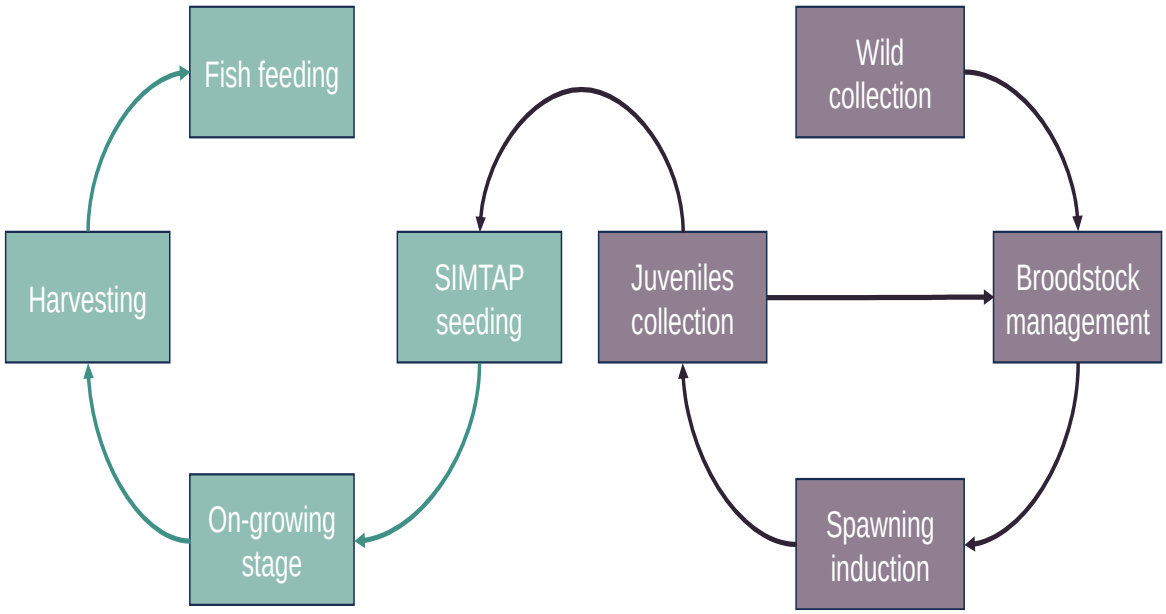

Fig. 19. Main stages and flows of polychaetes production: nursery and hatchery stage (right), on-growing stage in SIMTAP (left).

8.2 Collection of wild worms

- Wild specimens of *N. diversicolor* can be collected by manually digging the portion of substrate in which they live usually first 8-10 cm (**Fig. 20**);
- worms must be gently separated from the substrate by washing with clean water and with the help of a sieve;
- worms are placed in a tank filled with clean water taken from the collection site and maintained with constant aeration for 24-48 hours, afterwards wounded or dead worms should be removed.

Fig. 20. Collection of wild specimens of *Nereis diversicolor* from the Canale dei Navicelli (Pisa, Italy).

8.3 Reproduction under artificial conditions

The application of the following protocol requires a dedicated nursery facility for the maintenance of broodstock and the production of juveniles (**Fig. 21**). The nursery requires specific equipment such as: an automatic controller of photoperiod and room and/or water temperature; blowers for water aeration; other components typical of a recirculating aquaculture system (e.g., pumps, mechanical filter, biofilters, UV disinfection unit etc.). Both aeration and photoperiod manipulation are recommended to provide optimal growing conditions to the worms.

1 Worms are reared in the nursery at a temperature of 16-18 °C, a salinity level of 16-18 g/L, and a water flow of 6 L/h in specific culture tanks (those used at the University of Pisa had the following dimensions: nominal volume 38 L, internal measure 0.40 x 0.30 x 0.32 m, surface 0.12 m^2, material food-grade HDPE) in a 10 cm-deep sand (0.4-2.0 mm granule size) layer, with continuous water aeration with airstones or tubes. Each tank is inoculated with worms with individual body weight of approx. 50 mg at a density of about 150 individuals/m^2. The photoperiod will be maintained at 16:8 L:D (1.05 Wm-2) photoperiod with moonlight during the whole nightime. Moonlight is mimicked using white light (0.02 W m^{-2}). Worms are fed minced mussels ad libitum on alternate days; before feeding, the sand surface must be cleaned by syphoning the residues and dead worms.

2 With the first appearance of mature worms (green color), the tanks are transferred to a cold room/cabinet at 6 °C and with 14:10 L:D (1.05 W m^{-2}) photoperiod and moonlight during the night, as previously described. Worms are kept in cold room for one week in recirculating and well-aerated water or with daily partial (33%) discharge of water in case of stagnant water, following the same feeding and cleaning procedures previously described.

3 The thanks are then transferred back to the room at 16-18°C with 14:10 L:D photoperiod and without moonlight. Generally, juvenile worms of small (<5 mm) and medium (5-10 mm) size can be collected, respectively, after the cold treatment *(***Fig. 22***)*.

Fig. 21. 3D sketch of the layout of the facility for the breeding of Nereis diversicolor set up at the University of Pisa: shelf (1); recirculating tank (2), filters (3), UV lamp (4), air compressor (5), rearing tanks (6), biofilter (7), LED lamp (8).

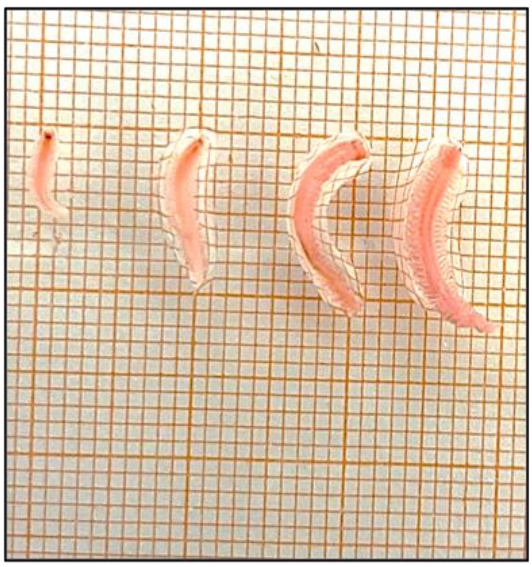

Fig. 22. Larvae of *Nereis diversicolor* with different sizes. From left: <5 mm, 5-10 mm, and >10 mm.

8.4 Worm culture in the SIMTAP

In SIMTAP polychaetes will be fed on suspended organic particles (SPOM) generated by fish. Thus, the polychaetes culture section should be dimensioned in order to assimilate all the solids generated by fish (i.e., feces and uneaten feed). Worms should be cultivated in specific containers (e.g., tanks, raceways, ponds) that should be designed to allow SPOM settling. The production of polychaetes can be conducted in both coupled and decoupled configuration. In the first case, worms receive the water coming from fish section with or without a previous mechanical filtration or suspended solid thickening (in this case, the suspended solids will naturally settle on the polychaetes growing substrate). In case of decoupled configuration, is recommended to feed the worms directly with the solids from filtering unit or after thickening (e.g., radial settler, clarifiers, etc.) in order to reduce the input of water in this section. The choice of the production method inevitably affects the complexity of this unit. Listed below the principal steps of worms production:

1. Juvenile worms are transferred from the nursery to the rearing unit at the desired density (250-2000 individuals/m^2). During the cultivation a 16:8 L:D photoperiod cycle (without moonlight simulation) is recommended to avoid uncontrolled worm reproduction.
2. The rearing unit should be designed to simulate as much as possible the natural environment of worms. A raceway-like system is recommended: low-depth substrate layer (8-12 cm) and water column 10-20 cm. This condition is favorable due to reducing the weight of the section (it can also be installed off soil for better handling), reduced water volume recirculating, and reduced risk of potential anoxic conditions in the substrate. Water should be well aerated before the introduction in the raceways/tanks. In the case of the decoupled configuration of this unit, a dedicated sump, biofilter, and thermal conditioning unit are recommended.
3. In case of diet integration (i.e., feed, discarded mussels, microalgae) water flow should be temporarily stopped to allow the sedimentation of all the suspended particles.
4. The trend of the worms' population is monitored by periodic (generally, every month) sampling to determine both the dimensions (length and body weight) and number.
5. Worms are generally collected after approximately four months from their introduction, when they reach a target size. In general, for *N. divesicolor* the size at harvest should be about 0.5 g.
6. Worms should be collected from the substrate once they reached the target size (**Fig. 23**).
7. Tanks or raceways should be emptied and worms collected from the substrate with the help of a sieve. Worms collected should be rinsed to remove all the sediment and SPOM debris.
8. In case of large system mechanical device can be built to help during the worms collection (see. https://www.youtube.com/watch?v=yOzAjtBrwIU).
9. After harvesting, worms can be frozen or dried according to the feeding administration adopted. A sample of the harvest should be analyzed for proximate composition and also for microbiological analysis. For instance, polychaetes can be vectors of the white spot syndrome virus (WSSV) in *Penaeus monodon* broodstocks (Bischoff et al., 2009). A sample of worms can be stored (better if freeze-dried) for further analysis. In case of use of dried worms in feed

production (e.g., tailored formulation), it is recommended a low-temperature drying up to 40°c max to preserve as much the nutritional profile of worms.

Fig. 23. Periodical collection of Nereis diversicolor in the culture tank by using pipes as sampling unit (left); the worms found in one pipe before counting and weighing (right).

9 Microalgae culture

9.1 Production of starter inoculum

The cyanobacteria or eukaryotic microalgae starting inoculums can be acquired from national and/or international microalgae collections.Alternatively, autochthonous strains can isolated autonomously in the laboratory associated with the SIMTAP system.

The national and international algae collections are very numerous; some of them are reported below:

Algae collections	City	Country
Maricoltura S.r.l.	Livorno	Italy
Tere Group	Modena	Italy
University of Padova	Padova	Italy
University of Milano	Milano	Italy
University of Pisa	Pisa	Italy
ACUF Algal Collection University Federico II	Napoli	Italy
CCAP Culture Collection of Algae & Protozoa UK	Oban	Scotland UK
NIVA Norvegian Institute for Water Research	Niva	Norvegian
NORCCA: The Norwegian Culture Collection of Algae	Oslo	Norvegian
SAG Culture Collection University of Gottingen	Gottingen	Germany
CCAC Culture Collection of Algae	Cologne	Germany
UTEX Culture Collection of Algae, University of Texas	Austin	Texas
Freshwater Algae Culture Collection	Hubei	China
Algal culture collections Bionity.com		Australia, Canada, France, Czech Republic, Germany, Mexico, Japan United Kingdom and United States.

Microalgae starters must be cultured in sterile glass tubes 810-25 mL) in liquid nutrient media or as algal smears on solid medium. To prepare an algae sterile inoculum in own laboratory, a sterility maintenance equipment is required.

At the University of Pisa, a *Chlorella*-like algal strain, named "SEC_LI_Chl", was used: it had been isolated in 2014 in a pond in Rosignano (Livorno, about 30 km far from Pisa), which collected the leachates from a municipal landfill. The *Chlorella*-like strain, named "SEC_LI_Chl_1", was identified and described through a multi-disciplinary study conducted in 2019-2020 (Ciurli et al 2021). The strain was cultured in laboratory in a modified Tris-Ammonium Phosphate (TAP) medium (Gorman and Levine, 1965) with the following composition of the growing medium: NH_4NO_3 0.285 g L^{-1}; $MgSO_4 \cdot 7H_2O$ 0.1 g L^{-1}; $CaCl_2 \cdot 2H_2O$ 0.05 g L^{-1}; K_2HPO_4 0.054 g L^{-1}; KH_2PO_4 0.054 g L^{-1}; and Tris 2.42 g L^{-1}. These microalgal strains were cultured and maintained in vitro in axenic conditions at 20±3 °C and 65-70% RH, with a photoperiod of 16/8 h day/night and a photosynthetic photon flux density (PPFD) of 70-100 µmol m^{-2} s^{-1}.

Under sterile conditions, the pure single colony from the tube supplied by the algal collections was placed to grow in small volume of sterile tubes or flasks (10-25 ml) and left in the growth chamber until the solution turns green. Keeping the sterility, culture volumes were gradually increased by adding new growth medium suitable until a suitable culture volume was obtained to start the microalgae culture in in photobioreactors (PBR), which was 10% of the capacity of PBR (100 or 20 litres; **Fig. 24**). For the maintenance of sterile algal cultures in the growth chamber, it is recommended adding fresh nutrient medium to the culture every 1-2 weeks.

Fig. 24. The photobioreactors used at the University of Pisa for the experiments with the Chlorella-like strain, named "SEC_LI_Chl_1": V-shaped plastic bags and vertical columns with a capacity of 100 and 20 L, respectively. In the bags, the microalgae were cultured in modified TAP medium (light green), greenhouse effluent (reddish) or artificial greenhouse effluent (dark green).

At the Mediterranean Fisheries Production and Training Institute (MEDFRI), Antalya, Türkiye, *Tetraselmis suecica* strain, a marine microalga, was obtained from the culture collection of MEDFRI. Monoculture of *T. suecica* was initially grown in 2 L flasks under a continuous light irradiance of 120 ± 20 µmol m^{-2} s^{-1} and a temperature of 21 ± 1 °C. Then flask's contents were transferred into plastic bag PBR of 80 L and the microalgae was grown to obtain stock under a continuous light irradiance of 90 ± 10 µmol m^{-2} s^{-1} and a temperature of 23 ± 1 °C.

Batch cultures in four AWW mediums processed by various treatments and Walne medium (**Table 10**) as control were tested in two different environments (**Fig. 28**). The first environment (was a culture room under continuous illumination of 90 ± 10 µmol m^{-2} s^{-1} from fluorescent tubes at the temperature of 23 ± 1 °C. The second environment was the culture unit in the SIMTAP prototype

where there was no control of illumination and temperature which changed depending on ambient conditions. The PBRs were continuously aerated with the blowers. Microalgae were cultured at indoor and greenhouse conditions for 20 days.

Fig. 25. The photobioreactors used at the Mediterranean Fisheries Production and Training Institute (MEDFRI), Antalya, Türkiye for the experiments with *Tetraselmis suecica* culture in marine aquaculture wastewater in the laboratory or in greenhouse.

9.2 Culture monitoring and biomass harvest

The microalgae growth parameters must be analysed at the beginning of the experiment and weekly for the whole duration of each assay and in all laboratories and/or greenhouse experiments are: optical density (OD), algal dry biomass production (DW) and pigment content (chlorophylls and carotenoids). The OD was measured spectrophotometrically at 530 nm, (Vona et al. 2004). The biomass was obtained by centrifugation of a volume (2-50 ml) of algal solution, the supernatant was eliminated, and the pellets were dried at 35°C until constant weight. The photosynthetic pigment extraction (chlorophyll a, chlorophyll b and total chlorophyll content) was performed in 100% methanol using the procedure described in Ciurli et al., 2020. Biomass is harvested by centrifugation (**Fig. 26**).

Table 10. Composition and preparation of Walne medium (https://www.ccap.ac.uk/wp-content/uploads/MR_Walnes.pdf)[2].

Stock 1	**Trace metal solution (TMS)**		
	$ZnCl_2$	2.1 g	
	$CoCl_2.6H_2O$	2.0 g	
	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.9 g	
	$CuSO_4.5H_2O$	2.0 g	
	Make up to **100 ml** with distilled water.		
	This solution is normally cloudy. Acidify with a few drops of conc. HCl to clarify.		
Stock 2	**Vitamin solution**		
	Vitamin B_{12}. (Cyanocobalamin)	10.0 mg	
	Vitamin B_1 (Thiamine.HCl)	10.0 mg	
	Vitamin H (Biotin)	0.2 mg	
	Make up to **100 ml** with distilled water.		
Stock 3	**Nutrient solution**		
	$FeCl_3.6H_20$	1.3 g	
	$MnCl_2.4H_20$	0.36 g	
	H3BO3		33.6 g
	EDTA(Disodium salt)	45.0 g	
	$NaH_2PO_4.2H_2O$	20.0 g	
	$NaNO_3$	100.0 g	
	TMS (1 above) 1.0 ml		
Medium	Nutrient solution (3) 1.0 ml per litre		
	Vitamin solution (2) 0.1 ml per litre		
	Sterilised seawater 1.0 litre		
	Dispense nutrient and vitamin solutions separately into 10 ml and 1 ml respectively and autoclave at 15 psi for 15 minutes. Add an aliquot of each aseptically to 10 litres of sterilised seawater.		

[2] Walne PR (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, and *Mytilis*. Fish. Invest. **26**, 1-62.

Fig. 26. A centrifuge to harvest microalgae biomass.

10 General prophylaxis and hygiene measures

10.1 Animal health and welfare issues

The occurrence of fish diseases usually results from the interaction among various factors (e.g., climate, nutrition, immune response, presence of pathogens, etc.) and are species- and system-specific. So far, specific aquaponic diseases have not been described but they may be similar to those in RASs. In aquaculture, non-infectious diseases are related with environmental, nutrition, and genetic factors; bacteria cause the largest part of infectious disease followed by viruses, parasites, and fungi. Fluctuations in water quality facilitates the development of fish pathogen. For instance, ubiquitous bacteria, which are present in any water containing organic matter, can become opportunistic pathogens under certain environmental conditions (Yavuzcan Yildiz et al., 2019). Nevertheless, the capability of a microorganism to cause a clinical disease depends on the interaction of several factors such as physiological status, host, husbandry, environment, and nutrition. The characteristics of an aquaponic system often reduce the risk of pathogen introduction and disease outbreaks due to better control of inputs and water management.

Since animals and vegetables grow together, the use of chemicals (e.g., disinfectants and antibiotics) should be limited to avoid microbial community dysfunction and the accumulation in plant edible tissues. Moreover, the use of antibiotics is not allowed in plant cultivation. Therefore, in aquaponics, more than in aquaculture, preventive measures should be applied to reduce the risk of infectious disease outbreak (Yavuzcan Yildiz et al., 2019).

Along with the use of pathogen-free water and stock, an important biosecurity measure is the quarantine for newly introduced larvae, fry or adult animals. Somerville et al. (2014) recommend a quarantine period of 45 days before the introduction of fish in the definitive rearing tanks. During quarantine, fish are checked and monitored for apparent disease signs and possibly exposed to

prophylactic treatments, for instance with vaccines for viruses and bacteria, salt bath for ectoparasites, or chemicals that do not leave residues in water.

Another way to prevent the spread of infectious and non-infectious diseases is the use of functional feeds or water enrichments. Several functional ingredients, such as probiotics and immunostimulants, can be added to formulated feed or directly in the rearing water. Among immunostimulants, glucans are the most studied and promising for aquaculture industry. Also, the addition of marine-derived polysaccharides in feed formulations is promising for their immunostimulants effect. Moreover, the extraction of these compounds (e.g., ulvan) could be integrated with the phytoremediation of aquaculture wastewater with seaweeds (e.g., Ulva spp.).

In soilless cultivation and RASs, UV irradiation is one of the most common disinfection methods. Continuous activity of the UV lamps and reduced load of suspended solids increase the efficacy of water treatment (Mori and Smith, 2019)..

A quick individuation of symptomatic fish is essential to prevent the transmission of infective agents and reduce fish mortality. Fish should be checked almost daily, looking for any un-usual behaviour (e.g., feeding and swimming activity) or lesions, then immediately removed from the rearing water and thoroughly inspected and transferred to a separate (quarantine) tank for any eventual treatment (Somerville et al., 2014; Yavuzcan Yildiz et al., 2019).

Comparing the two aquaponic design, on-demand coupled systems allow a better monitoring and the use of conventional treatments for fish disease that are not possible in permanently coupled systems.

Animal welfare

Fish welfare represents the physiological, behavioural, and cognitive responses, as consequence of stressful stimuli (Yavuzcan Yildiz et al., 2017). In a confined environment, such as aquaponic systems or RAS, the appearance of stressful stimuli results from the interaction of several factors: stocking density (i.e., the biomass of fish reared in a certain volume of water), water quality, diet, management procedures, and system design. These factors can increase the level of stress, negatively affect animal health, and induce the development of impaired or aggressive behaviour (Espinal and Matulić, 2019; Yavuzcan Yildiz et al., 2017). These conditions have a negative impact on the whole aquaponic system. Moreover, assuring a proper fish welfare can be an important driver for the consumers' acceptance of aquaponic products (Miličić et al., 2017).

Stocking density is a pivotal factor affecting fish welfare in aquaculture, especially in RASs where high densities are generally used to increase fish productivity (Espinal and Matulić, 2019). In general, the recommended fish stocking density is much lower in aquaponics than in RAS; for instance, it is 20 kg/m^3 in small-scale aquaponics (Somerville et al., 2014). In an aquaponic system rearing European Carp (*Cyprinus carpio*), an initial stocking density of 2.5 kg m^{-3} increased the rearing water quality and the yield of catalogna chicory, lettuce, and Swiss Chard, compared with a density of 4.6 kg m^{-3} (Maucieri et al., 2019). A reduced growth of fish raised at high density was also associated with higher TAN level and reduced DO concentration (Maucieri et al., 2019). The effect of different stocking densities (3.81 kg/m^3 and 7.26 kg/m^3) were also evaluated in rainbow trout (*Oncorhynchus*

mykiss) (Birolo et al., 2020). Water parameters (DO, pH, temperature, TAN, and nitrite) and fish growth were not affected by stocking density while the presence of *Pseudomonas* and H_2S-producing bacteria on fish skin increased with fish density (Birolo et al., 2020). The effects of sub-optimal water parameters on fish welfare are summarized in **Table 11.**

Table 11. The effect of non-optimal values of some water parameters on fish welfare in aquaponics (Yavuzcan Yildiz et al., 2017)

Parameter	Negative effects
Low DO content	Increased rate of opercular ventilation; gasping mouths; greater nitrite toxicity; reduced swimming activity; reduced growth; low feed efficiency; death
High pH	Increase un-ionized form of ammonia
High temperature	Infectiousness of many pathogens; toxicity of dissolved contaminants; reduction of DO level
High TAN content	Neurological disorder (acute); decrease survival; reduced growth; appearance of physiological dysfunctions
Nigh nitrite content	Conversion of haemoglobin into methaemoglobin; increased rate of opercular ventilation; morphological and physiological alterations of gills; hypoxia
Nigh nitrite content	Reduced swimming activity; decreased survival; reduced growth
High CO_2 content	Hypercarbia; moribund fish; gaping mouths; bright red gill lamellae

Fish raised in RAS and aquaponic systems require good water quality conditions, in particular regarding DO, CO_2, TAN, nitrite, nitrate, and pH. Carrying capacity represents the highest fish biomass with acceptable water quality (Yavuzcan Yildiz et al., 2017). Basically, the carrying capacity of a system is determined by the oxygen consumption by the fish and the accumulation of TAN, CO_2, and other potentially toxic metabolic wastes (Yavuzcan Yildiz et al., 2017). In an aquaponic systems, the carrying capacity of the water is one of the major concerns for maintaining the balance between plant and fish without negative effects on fish welfare.

Both plants and algae release chemicals compounds (allelochemicals) to make a more favourable environment to themselves (allelophaty). So far, negative effects of potential allelochemicals excreted by aquaponically grown plants on fish have not been reported (Yavuzcan Yildiz et al., 2017). However, algae (blue-green algae) can release cyanotoxins, which can directly affect fish health, and the algal spikes of diatoms (e.g., *Chaetoceros* spp.) can damage the gills (Chalmers, 2004; Yavuzcan Yildiz et al., 2017).

Another critical issue regarding water quality is the accumulation of solids. As already mentioned, solids should be removed as soon as possible, to avoid severe consequence on other components of

the system. The presence of an excess of suspended solids in the water is a stressful factor for the fish and can affect growth performance and increase the risk of diseases. Suspended solids can damage fish directly (e.g., damages to gills epithelium or smothering (Yavuzcan Yildiz et al., 2017).

Fish feed has a high impact on the carrying capacity of aquaponic water from a quantitative (feeding rate) and qualitative (feed composition and digestibility) viewpoint. Fish feed needs to fulfil fish and plant nutrient demand in aquaponics (Robaina et al., 2019). Most aquaponic systems use specific feeds formulated for RASs (Yavuzcan Yildiz et al., 2017), since little is known on specific formulations for fish reared in aquaponic systems. Specific supplementation is required for some micronutrients in aquaponics since the amount excreted by fish is generally inadequate to meet plants demand (Yavuzcan Yildiz et al., 2017). Tailored feed formulation for aquaponics need higher content of plant nutrients or making them more bio-available after excretion and biotransformation in fish (Robaina et al., 2019). Fish welfare can be directly affected by this practice. Since fish can take up micronutrients both from water and feeds, understanding the effect of excess of feed and waterborne micronutrients on fish and plants is crucial (Yavuzcan Yildiz et al., 2017).

Another factor affecting fish welfare is noise, which is generated by water pumps, blowers, and filtration systems. Noise can determine a stressful condition for the fish (Espinal and Matulić, 2019). Several studies have shown that fish behaviour and physiology can be negatively impacted by background noise and intense sound (Espinal and Matulić, 2019). However, these findings should not be generalized across farmed fish species, because many of them (e.g., catfish and cyprinids) have much greater hearing sensitivity than others (e.g., rainbow trout) (Espinal and Matulić, 2019). No significant differences were observed in growth rate, feed conversion, and survival of rainbow trout after five months of exposure to different noise levels (Davidson et al., 2009).

Table 12. Example of scheme for "Feeding Behavioural Scoring" related to feed conversion rate (FCR) and daily growth rate (DGR) of fish (Fronte, data not published).

Score	Behaviour	Description (Standard behavior of the fish species must be considered)	Note
5	Scarse appetite	Low interest in feed, partially consumed	FCR too high
4	Pretty low appetite	Feed consumed almost totally; fish not very "mobile"	Best DGR
3	Regular appetite	Totally consumed feed, "interested" and "lively" fish	Best FCR
2	Pretty strong appetite	Pronounced fish competition for feed	FCR e DGR sub-optimal
1	Starving fish	Strong competition for feed, pretty violent fish behavior	High FCR, low DGR

Feed consumption and feeding behaviour are among the most important indicators of fish welfare status. For these reasons it is suggested to "score" the fish feeding behaviour (**Table 12**) on a daily base. Possibly, the observation and behaviour scoring should be led always by the same operator (trained) and at same time of the day (e.g., first meal of the day). In this way, the operator can adjust the amount of feed daily supplied according to the observed behaviour of the previous days (from 1 to 3 days).

10.2 Food safety

Foodborne illnesses are significant hazards for human health. Human pathogens and chemical contaminants must be maintained within safe limits or completely prevented (Jennings et al., 2016). Food safety risks exist in integrated food production systems and aquaponics is not an exception.

In aquaponics, the main concern is due to the copresence of aquatica animals and edible plants grown in recirculating water, which could provide suitable conditions for the development and spread of pathogens (Mori and Smith, 2019). Special attention should be paid for vegetables that are usually consumed raw, in particular for leafy vegetables and herbs that grow closer to the recirculating water than fruity plants, and then can be easily contaminated.

Aquatic animals are not considered high probability vectors of zoonotic diseases to humans, due to significant physiological differences between them (i.e., fish are cold-blooded animals while humans are warm-blooded mammals). However, several microbiological agents (e.g., *Streptococcus spp.*, *Aeromonas spp., Listeria monocytogenes, Escherichia coli, Salmonella spp., Vibrio sp*p., and others) were found to trig diseases in humans and hence to endanger the safety of aquaponic products if a contamination occurred. Shiga toxin-producing Escherichia coli was recently isolated from fish feces, water, and on the surface of the roots of lettuce, basil, and tomato without internalization in edible portions (Wang et al., 2020). Possible sources of bacterial pathogens are water, fish feed, fish juveniles introduced in the system, plant propagation materials, and humans themselves (staff and visitors). Therefore, strict hygiene measures should be adopted in aquaponics. The young plants introduced in the system must be free of coliforms and other human pathogenic bacteria. Staff is required to wear gloves when handling fish, paying attention to avoid spines and to work with open wounds. Working with bare hand could introduce new pathogens in the system. Harvesting equipment and other tools have to be kept clean and sanitized. Plants products should be harvested avoiding the contact with water; nevertheless, it is advisable washing any aquaponic products before consumption (Somerville et al., 2014).

In addition, proper practices for solids management are required, and fish feces should be continuously removed from the system to limit the release of coliform bacteria (Somerville et al., 2014). Even diseased fish or plants should be promptly removed, to hamper the subsequent shedding of potential pathogens in the water (Mori and Smith, 2019).

The microbiological quality of the water in aquaculture and hydroponic systems can be ensured using physical disinfection and filtration methods, such as UV, ozone or hydrogen peroxide treatment, blue light-emitting diodes (LED), heat, sonication, media or membrane filtration (Mori and Smith, 2019).

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