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SMART AQUAPONICS
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Smart Aquaponics Guide : Fish production

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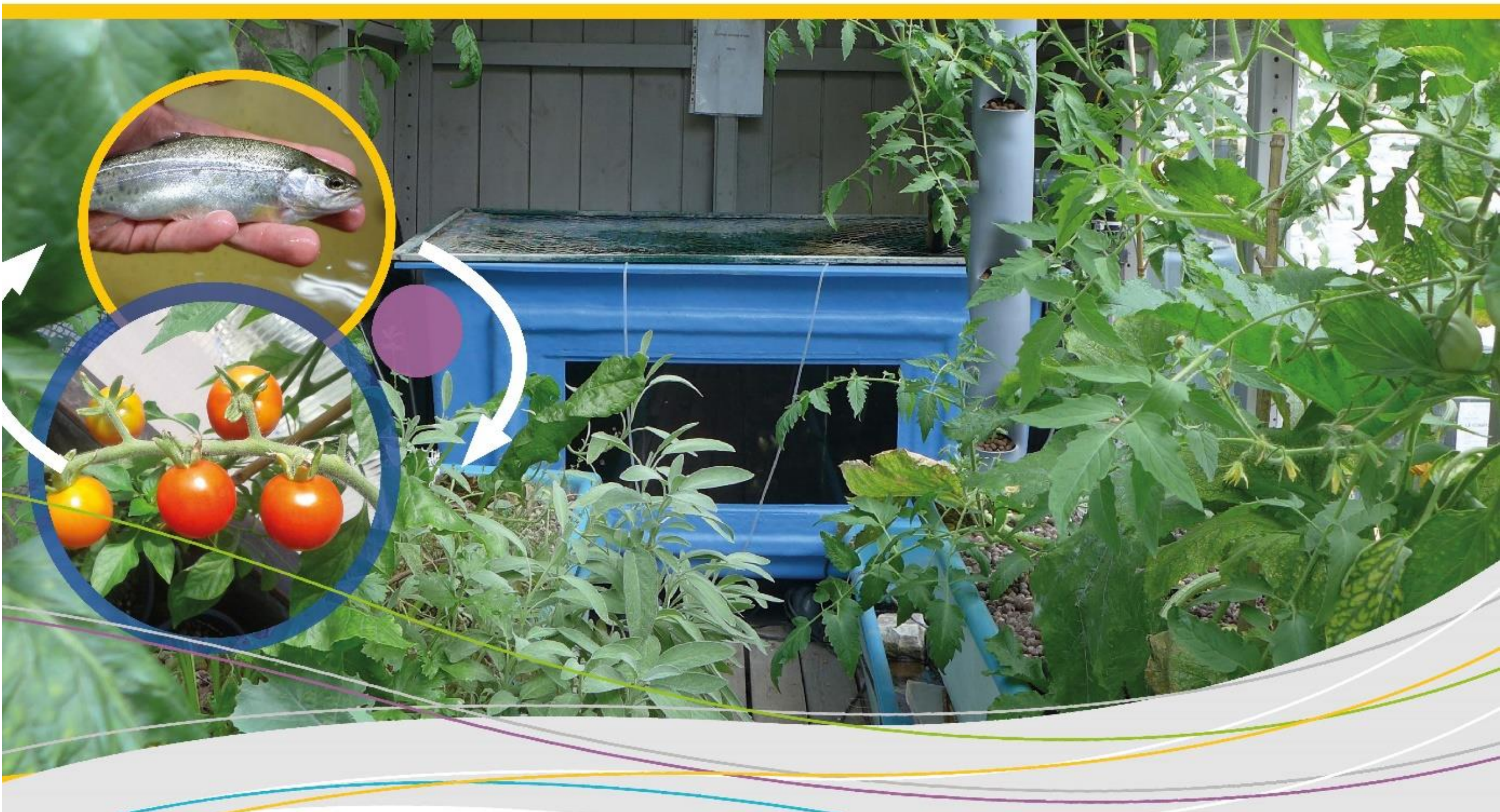

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Preamble:

This document relating fish and aquaculture system description is fully based on the aqu@teach textbook (Jungle et al. 2020). The complete textbook is available at http://doi.org/10.5281/zenodo.3948179.

1 Recirculating Aquaculture Systems

1.1 Introduction

Aquaculture is the captive rearing and production of fish and other aquatic animal and plant species under controlled conditions. Due to overfishing and the consequent decline of wild fish stocks, aquaculture has become increasingly important in the past few decades (Figure 1), and may become even more so in the future as wild fish stocks face immense pressure from climate change.

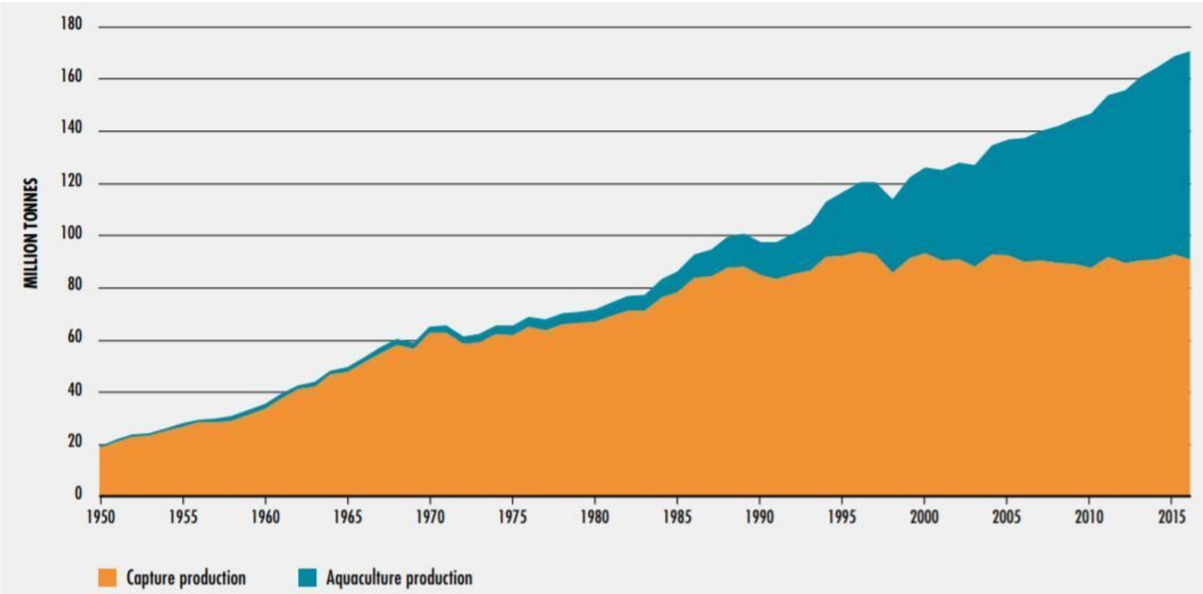


Figure 1: In 2016 aquaculture accounted for around 47% of total global fish production (FAO 2018)

The main goal of any aquaculture system is to produce, grow and sell aquatic organisms such as fish, crustaceans, molluscs, algae or seaweed. In this course we will focus on the aquaculture of fish. The basic situation of fish rearing is shown inFigure 2. Fish living in a water body receive feed and oxygen. Their metabolism converts these into excreta and CO2 which, if they accumulate in the water, are toxic for the fish. Different fish farming technologies cope with this problem using different strategies.

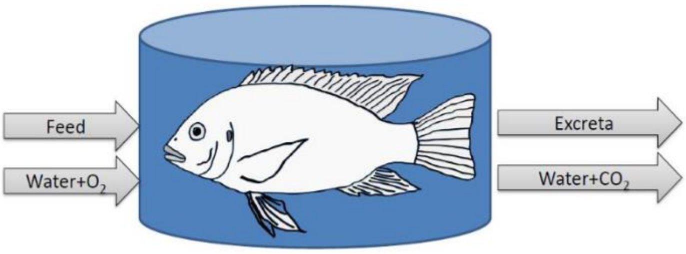


Figure 2: The basic principle of aquaculture from a water perspective. Fish living in water receive feed and oxygen. Their metabolism converts these into excreta and CO2, which are toxic for the fish. The water becomes waste water

Aquaculture systems can be classified into four basic types: fish ponds, net enclosures, flow-through, and recirculation systems (Figure 3). 'Open' aquaculture techniques such as net enclosures, pond cultures, and flow-through systems release nutrient-rich wastewater into the environment, potentially causing eutrophication and hypoxia in water bodies. In recirculating aquaculture systems (RAS) this waste water is treated and re-used within the system.

RAS has several advantages when compared to other aquaculture systems: it is a totally controlled system that is largerly independent of local conditions; it has very low water usage with low wastewater flows; and production can be planned and targeted year-round. However, there are also disadvantages, such as significant investment and operation costs, and high operation risk due to failure-prone technology. Species selection is limited mostly to carnivorous species, and the system is utterly dependent on artificial feeds. In this context, aquaponics can be viewed as a form of RAS or an extension of RAS. Therefore, in this chapter, the aquaculture part of a recirculating aquaponic system is presented in more detail.

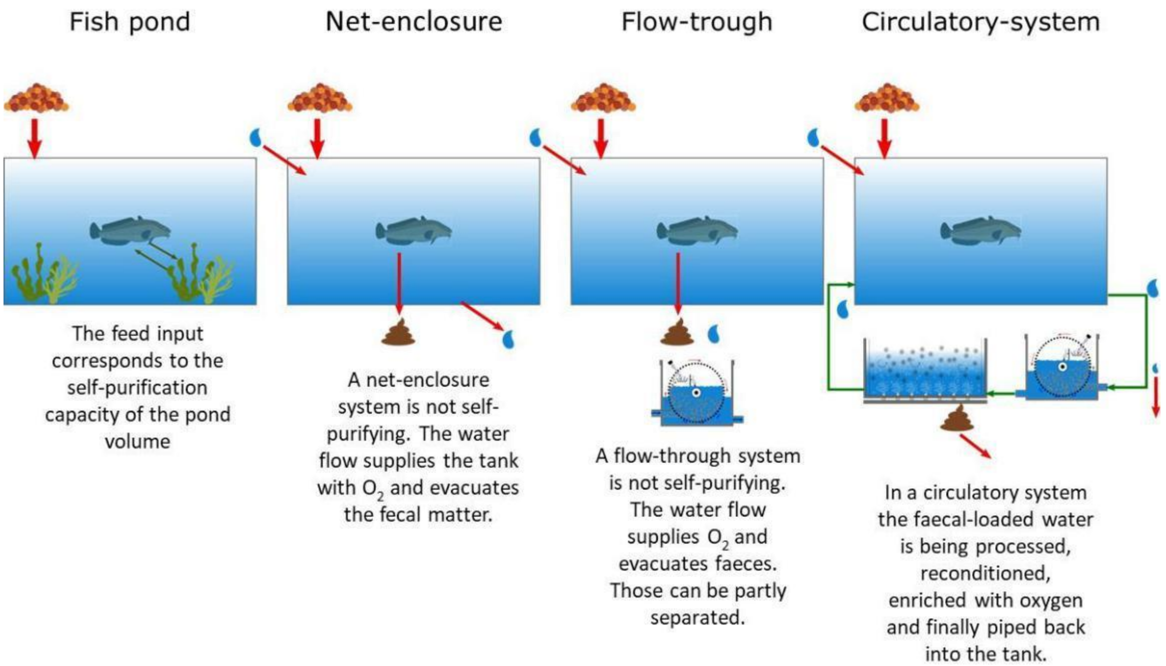


Figure 3: The main types of aquaculture systems

1.2 Recirculating aquaculture system (RAS) technology

A recirculating aquaculture system (RAS) consists of fish tanks and several filtration units which clean the water. In a classic RAS the water is thereby in constant flow from the fish tanks through the filtration system and then back to the fish tanks (Figure 4). Due to the metabolism of the fish, the water that leaves the tanks contains high concentrations of solids, nutrients, toxins and carbon dioxide, whilst it is oxygen-poor compared to inflowing water. The goal of the filtration units is to decrease the solids, nutrients, toxins, and carbon dioxide concentrations, and increase the levels of dissolved oxygen in the water before it is returned to the fish tank. The filtration system consists of several stages (Figure 4). The first treatment step after the outflow is the solids separation (Figure 4, Point 2) where the solids (feed remains, faeces, larger algae and bacteria assemblages) are removed from the water. After this, the water is disinfected with UV or ozone (Figure 4, Point 6). This step is not always implemented in fish farms and can also be placed after the biofilter. The water then enters the biofilter (Figure 4, Point 3), where bacteria metabolise part of the organic load, and oxidize ammonia to nitrite and then to nitrate. All these bacterial metabolic reactions use dissolved oxygen (O2) and release carbon dioxide (CO2) into the water. Therefore, the CO2 levels in water have to be lowered after biofiltration. This is done in the degassing unit in which the water to air surface area is increased so that the CO2 enters the air phase (Figure 4, Point 4). As a last step, the oxygen concentration in the water has to be increased to a suitable level for the fish. This is done in the oxygenation unit (Figure 4, Point 5). The following sections describe these system components in more detail.

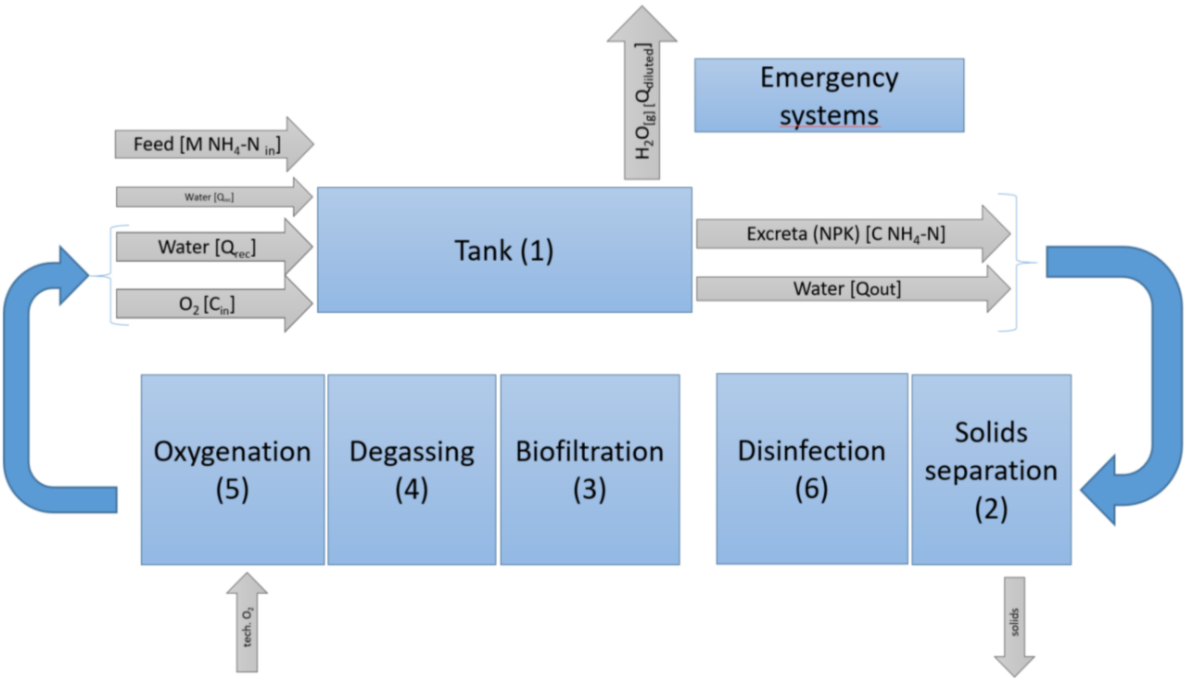


Figure 4: Main components of a recirculating aquaculture system (RAS)

1.2.1 The fish tank

1.2.1.1 General

The fish tank is the grow-out area for the fish and therefore a core component of a RAS. This tank will be the home of the fish for a relatively long period of time, so it should be chosen with care. The materials, design and size of the fish tank are all important, and should enable relatively easy observation and handling of fish, removal of solid particles, and good water circulation (simulation of natural water flow).

1.2.1.2 Design

1.2.1.2.1 Volume

The volume of the fish tank depends on the following factors: (i) the number of fish it will have to house, (ii) the volume of the living space that each fish species requires, and (iii) the method of maintaining a stable water temperature. The design of aquaponic systems is based on the quantity of fish feed, which is related to fish density. The required volume of the fish tank is based on targeted fish density and biomass. For example, if the target density is 10 kg/m^3, and it is planned to cultivate 30 kg of fish, a 3000 litre fish tank will be needed. One must also be aware that the fish will grow, and therefore the fish density and biomass will also increase during the production cycle. Generally, larger systems are more stable in terms of water temperature oscillations.

Figure 5: The importance of fish tank volume for water temperature oscillations: (left) small fish tanks exhibit faster water temperature changes; (right) in larger water volumes the temperature will be more stable.

1.2.1.2.2 Shape

The ‘classic’ tank designs are round and rectangular tanks. One of the main aspects that makes round tanks favourable over rectangular tanks is the self-cleaning effect that can be achieved through a circular hydraulic pattern. The flow in the fish tanks has two functions: (i) uniform distribution of inflow water and fish feed; and (ii) transport of particles to the centre of the tank. Primary rotating flow is the flow from the inlet and then clockwise/anticlockwise around the tank. It transports settleable solids to the bottom. The primary rotating flow creates secondary radial flow and together they generate a self-cleaning tank.

Figure 6: Different forms of fish tanks: (left) circular tank, (centre) rectangular tank (raceway or plug flow), and (right) double-D tank or D-ended raceway (hybrid of circular and raceway) (source: www.aqua-tech.eu, Bregnballe 2015)

In round tanks the water and dissolved nutrients as well as the particles have a relatively short residence time compared to square tanks due to the hydraulics. Although round tanks have numerous advantages over square tanks, their main disadvantage is low area efficiency, which often makes them a suboptimal solution for a RAS farm. Therefore, numerous other forms of tanks have been developed and tested in the past decades, e.g. the double-D or endless tank (Figure 6)

Table 1 summarizes some general advantages and disadvantages of round, square and double-D tanks. In addition to these, other factors need to be considered, such as the type of fish species that one wants to rear. Bottom-dwelling fish such as burbot, turbot, sole or similar flatfish mostly stay on the bottom of the tank and may prefer a slow waterflow. Moreover, the bottom-dwelling fish may be stocked in such a way that the self-cleaning of the tank is actually achieved through fish movements and not the hydraulic pattern of the water column. Therefore, a square tank design may not be the worst solution for farming bottom-dwelling fish. Another aspect of tank design is the inclination of the tank bottom. While it has very little effect on the self-cleaning ability of the system, a higher inclination may help with draining the whole tank.

Type of fish tank	Advantages	Disadvantages
Circular	• Structural stability, no pressure points on corners • Less material needed (cheap tank equipment cost) • Conceptually simple • Allow for homogeneous distribution of water and good water quality • Flow conditions (centrifugal forces) wash the sediments towards the outflow in the centre of the basin centre towards the outflow (high self-cleaning effect) • Low residence time of particles • Oxygen control and regulation easy	• Low area efficiency, low space utilization • Hard to seal tank connectors (pipe through tank wall) • Hard to segment • Flow rates vary within the in tank
Square	• Efficient usage of area and space • Easy to seal tank connectors • Simple segmenting • Easier to handle the fish	• Low self-cleaning (possible dead zones, concentration gradients of dissolved oxygen and ammonia emerge) • To prevent low self-cleaning high flow rate needed

		• High residence time of particles • Medium oxygen control and regulation • Pressure points in structure • Feed waste is higher due to greater dispersion of the fish
Double-D	• Efficient usage of area and space • Water mixing partly possible • Simple segmenting • Medium self-cleaning • Oxygen control and regulation easy • The fish can swim in circles	• Conceptually complex • High amount of materials needed More expensive

Figure 7: Advantages and disadvantages of round, square and double-D fish tanks

Since RAS has gained popularity and these systems are also planned as large-scale ventures (e.g. Nordic Aquafarms is planning to invest in a 500 Million USD RAS farm in Belfast, Maine, USA), large tank designs have become increasingly important. These large tanks are often (at least in theory) much more cost-efficient than the traditional smaller tanks (Figure 8).

Figure 8: A large round tank (6 m deep, 32.5 m in diameter) as part of a salmon RAS (Swiss Alpine Fish)

1.2.1.2.3 Water flow

The flow in the fish tanks has two functions: (i) uniform distribution of inflow water and fish feed; and (ii) transport of particles to the centre of the tank. Primary rotating flow is the flow from the inlet and then clockwise/anticlockwise around the tank. It transports settleable solids to the bottom. The primary rotating flow creates secondary radial flow and together they generate a self-cleaning tank.

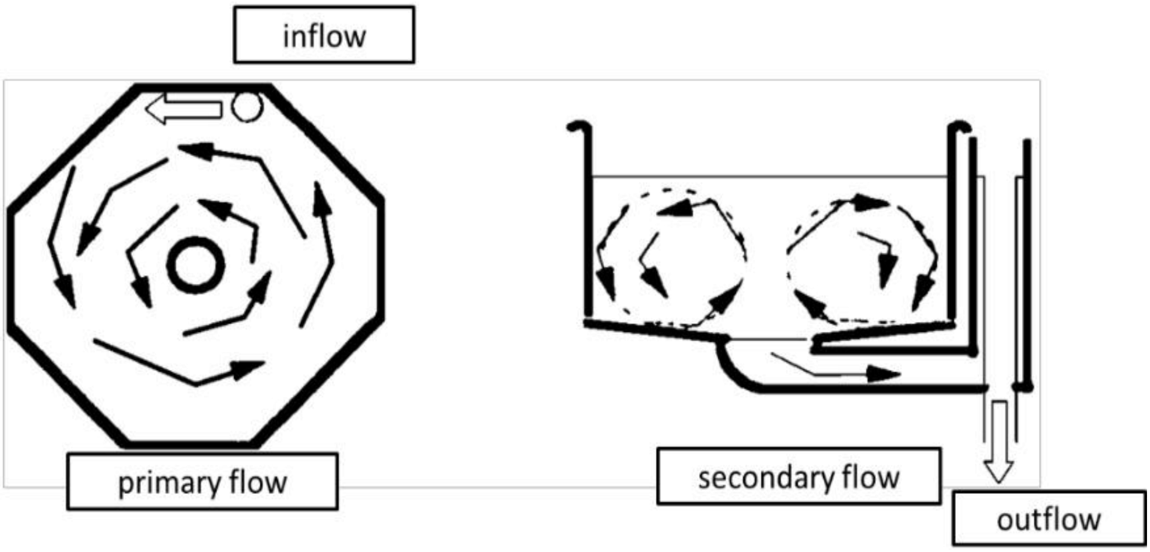


Figure 9: Role of primary and secondary flow patterns: the primary flow ensures good water distribution of the inlet water and the secondary flow contributes to effective solids removal (adapted after Timmons et al. 1999)

Flow conditions have an important impact on fish health. One can establish different water flows and thus structure the basins hydraulically by using panels. In this way the fish stay in the optimal part of the tank.

It is important to know that swimmers need to swim, in other words they need a current. The speed of the current must be adapted to the fish species. Generally, smaller fish require a lower current speed, though it must be high enough to ensure that the solids separation still works. All this also has an impact on the quality of the fish flesh.

1.2.1.2.4 Inflow and outflow

Ideally water should flow into the tank at an angle from above in order to enrich the water with oxygen and generate a circular flow in the tank (Figure 10). If you the water is oversaturated (oxygen saturation >100%, caused by the oxygenation units such as a low head oxygenator or oxygen cone), then the water should enter the fish tank below the surface through a perforated pipe (flute) which creates a circular water flow. The first perforation should lie just above the surface of the water and the total cross section of all perforations in the inflow pipe should be equal to the pipe’s cross section. The perforations also need to be smaller than the size of the fish is kept in the system.

Figure 10: Examples of water inflow and outflow: (left) the water inflow is located above the tank at an angle; (right) the water outflow is in the centre of the bottom of the tank photos: U.Strniša)

The outflow of water from the tank should enable the removal of solid particles, while at the same time preventing the loss of fish; it is therefore usually placed in the centre of the bottom of the tank (Figure 12). The correct dimensioning of the system and water flows prevents both clogging and overflowing. Each fish tank should be built as a separate hydraulic element, since hydraulic communication between fish tanks will end in total loss of all the fish if one pipe or one tank leaks. Therefore, every tank needs an option for overflow. Normally the tank has external standpipes or external overflows, so that structures within the fish tank do not interfere with fish handling procedures.

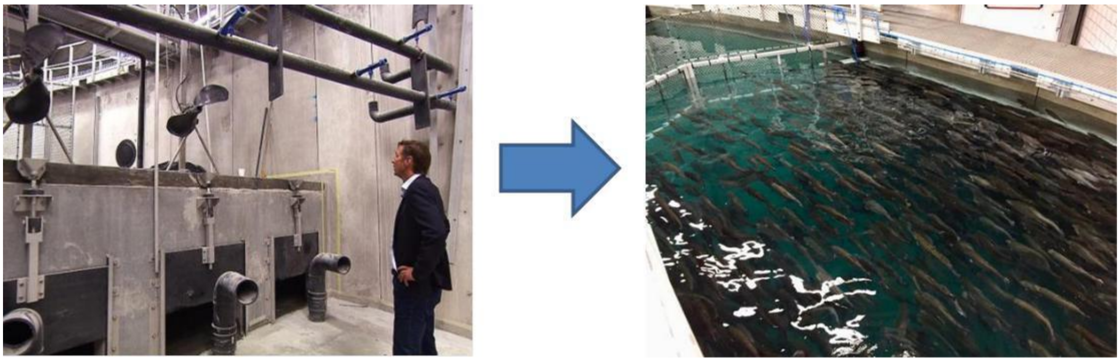


Figure 11: Flow system specially developed for farming salmon, Swiss Alpine Fish AG, Lostallo, Switzerland

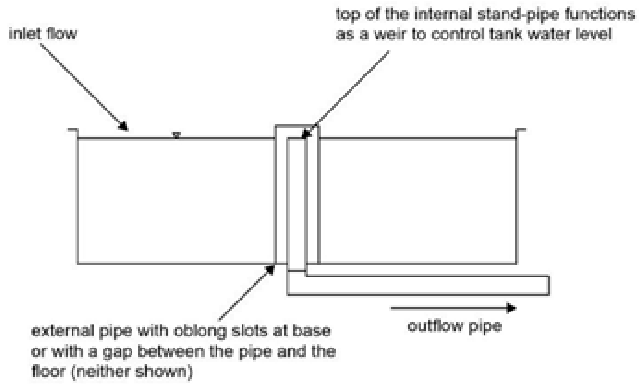
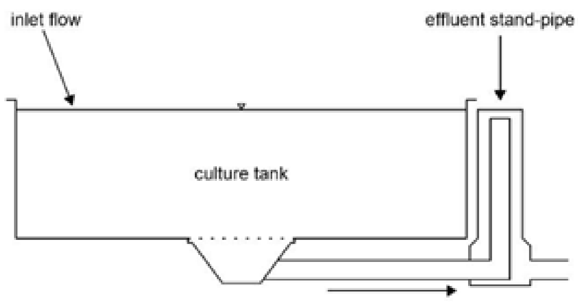
Type	(+) Advantages / (-) Disadvantages	Section
internal standpipe	(+) Water level control (+) No sediment deposition in pipeline (-) Disturbs netting of fish	
External standpipe	(+) Water level control (+) Tank free of installations (-) Solids can settle in the pipe segment	

Figure 12: Water outflow options (Source: Timmons & Ebeling 2007)

1.2.1.2.5 Height and ratio

The fish tank should be at such a height that it allows staff to observe and work with the fish. If using deeper tanks, a window for observing the fish should be included and/or a stable walkway to access the tank. The height of the tank also determines the height of the water column and the rate of water flow to the next component of the aquaponic system.

Figure 13: Fish tanks positioned (left) above ground (photo: U.Strniša), and (right) at ground level (source: www.humblebynature.com/about-us/projects-at-humble- by-nature/aquaponics-solar-greenhouse)

If you are using a circular tank, you have to make sure that the water diameter/height follows a certain ratio. The maximum ratio should be 6:1. If the tanks are wider, then solids removal and even distribution of water from the inflow will be hindered. Reducing the ratio below 3:1 will create a vortex in the central drain, and oxygen will not be distributed evenly in the tank. Ratios below 3:1 should include a side drain (dual drain) to avoid the build-up of a vortex.

1.2.1.2.6 Materials

There are differences regarding investment costs, tank stability, and installation, but the most important thing is to make sure that the materials are safe for both the fish and the plants. This means that galvanized materials should be avoided, because of zinc toxicity. The wrong type of plastic can also be harmful to the fish. Thermally weldable plastics (so called thermoplasts such as PE, PP or PVC) are the best option, though they tend to be more expensive. The choice of plastic needs to take into account the following considerations:

- UV resistance (black PE is UV resistant)
- Porosity (PP is more porous than PE and therefore enables biofilms to grow)
- Thermal stability (PVC becomes brittle below 0°C)

Because of its resistance to hard weather conditions, PE is the material to choose for long-lasting installations in greenhouses or outdoors.

Figure 14: Different fish tank materials: (top left) polyethylene (photo: U. Strniša), (top right) concrete (photo: U. Strniša), (top right) concrete (photo: U. Strniša), (bottom left) steel tanks covered with plastic liner (photo: ZHAW), and (bottom right) PVC tanks

1.2.1.2.7 Tank cover

Healthy fish are lively creatures and can jump out of the tank. All tanks should therefore be covered in order to prevent accidental losses and injury to the fish. Covers also prevent foreign objects from falling into the tank (Figure 15). Tank covers reduce water losses due to evaporation and provide shading, which reduces overheating, prevents algae growth, and thus improves the wellbeing of the fish. In addition, most fish prefer to be in the shade rather than in direct sunlight.

Figure 15: (left) A fish tank covered with netting to prevent accidental losses; (right) A tank liner and planted rafts prevent algae growth and provide shade (all photos: U. Strniša)

1.2.2 Solids separation

1.2.2.1 General

There are several reasons for the removal of solids. Firstly, water quality is improved by reducing the organic solids which reduces mineralisation (aerobic respiration) and therefore also helps to stabilize the oxygen content. Secondly, the preservation of the water quality also benefits feed uptake and stock control. Furthermore, solids removal reduces the bacterial load, because it removes the food source for microorganisms. High bacterial activity in the water column leads to unnecessary consumption of oxygen.

Another benefit of solids removal is the prevention of clogging of the fish gills which may lead to slow growth or even fish death. However, this depends on the fish species. Filter feeding fish, like many carp species, may even rely on a certain amount of suspended compounds in their natural habitat and can therefore also withstand a higher amount of suspended solids in RAS than, for example, salmonids (Avnimelech 2014).

One of the most important technical reasons why solids need to be removed is the potential clogging of the biofilter. Moreover, the effectiveness of germ reduction through disinfection is increased through the removal of solids. The solids in fish water have different sizes, and treatments to remove these solids vary mostly according to their size (Figure 16).

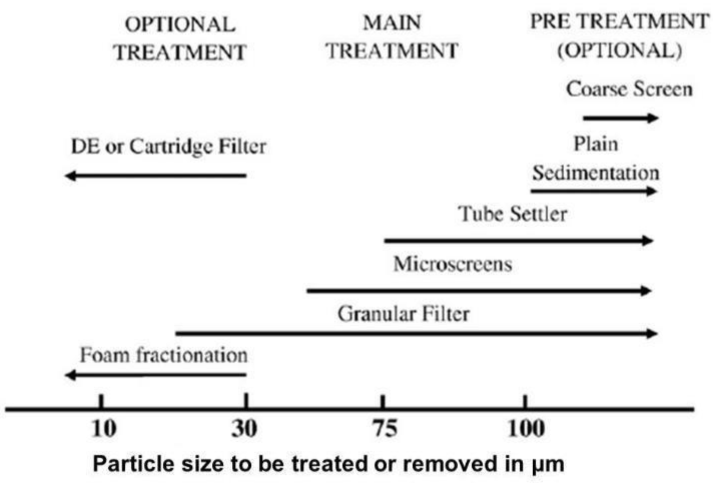


Figure 16: Solid removal processes and the particle size range (in µm) over which the processes are most effective (after Timmons and Ebeling 2007)

Wastewater treatment is an important cost factor of intensive RAS. This is normally charged per m^3 of wastewater that leaves the facility and enters the public wastewater plant. To minimize the volume of wastewater it is feasible to treat the sludge water that results from the solid separation.

1.2.2.2 Design

The following decisions need to be made during the design stage:

1. Is a separate solid removal step necessary?

In systems with a low fish stocking rate, a media growing bed can remove solids and act as a biofilter. However, over time, clogging and anaerobic areas will occur as the amount of solids increases.

2. What is the appropriate device for solids removal?

Waste particles in the water can be of different sizes, which affects the technologies used to remove them. Systems with a lower stocking density (<10 kg/m3) may be able to use devices based on sedimentation for particle removal, while systems with a higher stocking density (>10 kg/m3) may need rotational drum filters.

3. How should the fish tank be connected to the solids removal device?

The water should always flow by gravity from the fish tank to the solids separator and not be pumped, since the latter will only decrease the particle size and make it more difficult to remove. To avoid sedimentation the flow velocity in the pipe should be between 0.7 to 1.0 m/s.

4. What to do with the sludge?

The sludge resulting from the solids removal can be managed in several ways. These are discussed in subchapter 2.2.8 Wastewater treatment.

Solids removal techniques

1.2.2.2.1 Sedimentation

Most low-tech systems use gravitational sedimentation for the removal of particles. Filters in this category are: vortex filter, lamella separator, and radial flow separator (Figure 15). The low-tech sedimentation filters can normally only cope with particles of a size larger than 100 µm. However, due to the high flow and active mixing of the water column, the majority of particles in most modern intensive RAS will be smaller than 100 µm. Therefore, using sedimentation filters only is not an optimal solution for intensive RAS.

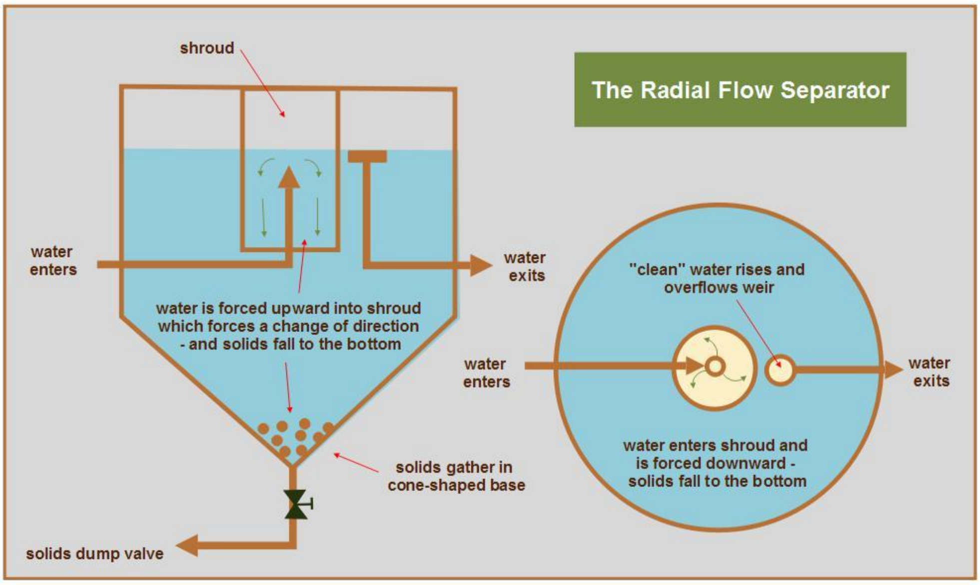


Figure 17: Diagram of a radial flow separator (www.garydonaldson.net)

1.2.2.2.2 Microscreens

Most modern and intensive RAS use microscreens, often applied as rotational drum filters for solids filtration (Figure 18). These drum filters work in the following way: water enters the drum filter and filters through the microscreens (usually with a filter cloth of 40-100 µm), solid particles are held back and then washed from the filter elements into the sludge tray, and the sludge water then leaves the fish system and enters the waste water treatment facility.

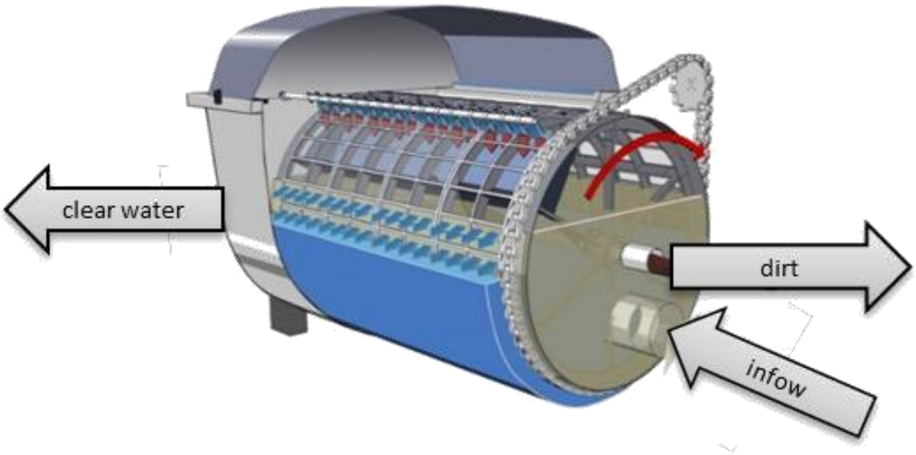


Figure 18: Diagram of a drum filter (www.nordicwater.com)

1.2.2.2.3 Foam fractionation

In addition to the drum filters, foam fractionators (also called protein skimmers) (Figure 19) are often used. These are mainly used to remove organic compounds such as proteins but they have also been reported to reduce a wide variety of other organic and inorganic molecules (e.g. fatty acids, detritus, bacteria, metals).

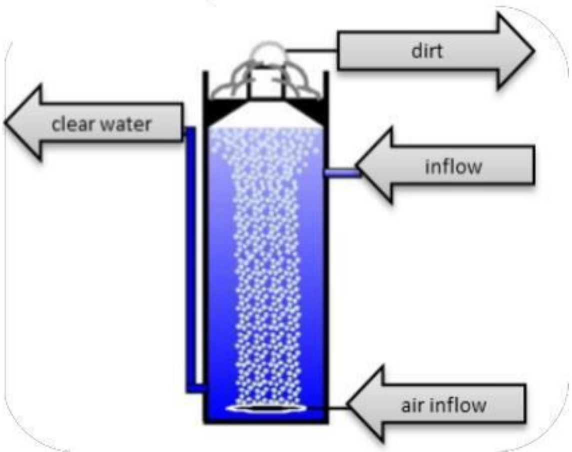


Figure 19: Diagram of a foam fractionator (www.epd.gov.hk)

	Sedimentation Filter	Drum Filter	Floating Filter
Principle	Density (gravity)	Filtration (size)	Flotation (polarity/density)
Size	>100 µm	>30-100 µm	<30 µm
Pressure drop	Insignificant	20 cm	Insignificant

Figure 20: Characteristics of different solids filtration systems

Figure 21: Different solids removal devices: (left) sludge trap; (centre) roughing filter; (right) rotational drum filter at ZHAW (all photos by U.Strniša)

1.2.2.2.4 Aquaponics

In systems with low fish stocking density, a media growing bed can take over the role of solids removal. If the solids load is too high, clogging and anaerobic areas can occur, which reduces the efficiency of biofiltration in the media. Therefore, if the growing bed is to function as a biofilter, either a very low fish stocking or a separate solids removal device are recommended.

1.2.3 Disinfection

1.2.3.1 General

Bacterial as well as viral diseases can pose serious problems in intensive RAS. Disinfection of the water using ozone or UV-irradiation are the most common methods.

1.2.3.2 Design

1.2.3.2.1 UV

UV light at a certain intensity can destroy the DNA of bio-organisms such as pathogens and single-celled organisms. In RAS the UV light (Figure 22) is mostly encompassed in a short piece of pipe between the mechanical filtration unit (e.g. drum filter) and the biofilter. The intensity or dose of UV light can be expressed in µWs/cm2 (energy per area). In RAS the UV dose needed to kill (deactivate) around 90% of the organisms ranges between 2000-10 000 µWs/cm2. However, to kill all fungi and small parasites a dose of up to 200 000 µWs/cm2 may be necessary. For maximum efficiency it is important to place the UV light after the mechanical filtration system so that it is not blocked by the suspended solids.

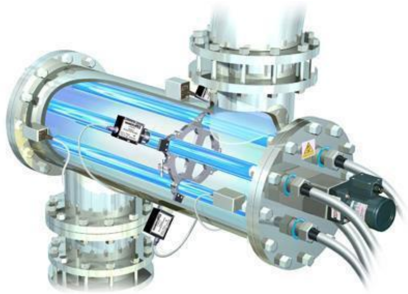


Figure 22: UV reactor (AKR UV Systems)

1.2.3.2.2 Ozone

The addition of ozone (O_3) is another efficient method for reducing pathogens and other unwanted organisms in a RAS. Ozone is an oxygen molecule (O_2) with an additional oxygen atom. In contact with water it splits into O_2 and a free oxygen radical O. This radical 'attacks' and oxidizes organic substances. This results in the degradation of suspended particles or some substances (clarification of water turbidity, colour formation by humic acids). Likewise, the biological cell walls of the organisms are also attacked by the radical O of the ozone molecule, killing off bacteria, floating, and filamentous algae. However, ozone is very reactive and can also harm the nitrifying bacteria in the biofilter and attack the fish gills if applied in too high amounts. The dosage therefore has to be monitored permanently.

Disinfection Agent	Advantages	Disadvantages
UV	- Works only locally in the UV - Reactor - Can be applied without harming fish - Simple management - Cheap	- Sensitive to water turbidity, ineffective in water with high solids loading - Bulbs need to be replaced (every year) - If the radiation period is too short (i.e. the system has a too high flow rate) the UV-disinfection is ineffective
Ozone	- Very effective in killing unwanted organisms like pathogens - Breaks down complex molecules into small, biodegradable compounds - Oxidizes nitrite to nitrate	- Complicated dosing - Can harm fish and biofilter - On-off of ozone system may lead to varying nitrite levels and decrease the amount of nitrifying bacteria in the biofilter - Relatively expensive -
H2O2	- Very effective in killing unwanted organisms like pathogens	- Limited application, like disinfection of empty tanks and equipment - Overdose is likely to severely damage the fish! - Also damages the filter

Figure 23: Advantages and disadvantages of disinfection with UV, ozone, and hydrogen peroxide (H2O2) in RAS

1.2.4 Biofiltration

1.2.4.1 General

The nitrification process takes place in the biofilter to oxidise the highly toxic free ammonia into less toxic nitrite and eventually to the non-toxic nitrate. The nitrifying bacteria are the heart of the biofilter. These bacteria grow on the filter media surface. The media can be fixed (e.g. trickling filter) or moving (e.g. moving bed filter). The nitrifying bacteria are sensitive to water quality changes in the system (especially pH and temperature), and rapid changes should therefore be avoided or done in slow steps as otherwise large amounts of nitrifying bacteria may die off which would lead to ammonia and nitrite spikes in the system. Moreover, as the nitrifying bacteria are aerobic, the dissolved oxygen content in the biofilter should always be kept at a certain threshold (depending also on the water temperature). As a rule of thumb the concentration of oxygen at the biofilter outflow should not be lower than 1 mg/l.

1.2.4.2 Design

The biofilter is the heart of every recirculating aquaculture system. Fish health, and therefore economic success, depend on correct operation of the biofilter. High ammonia and nitrite levels in fish tanks can be caused by several factors. One of the these can be poorly designed or sub-optimal operation of the biofilter (too small, not mixed evenly, nitrate levels too high, pH too low, intoxication of the biofilter by salt or medical treatment, aeration too low or too high, etc.). The other main aspect of design failure is insufficient recirculation of the water. The biofilter can only degrade what it receives from the fish tank. If the recirculation rate is too low, even an over dimensioned biofilter will not lead to good water quality.

1.2.4.2.1 Choosing the biofilter

Moving bed biofilter reactor (MBBR) is the most commonly used biofilter type in aquaponics and in RAS (Figure 24). The media of a moving bed filter consists of small (1-2 cm) plastic structures with high specific surface area (e.g. Kaldness k1). This filter media is kept in constant movement by aeration (e.g. through input of air through air-plates at the bottom of the biofilter tank). The constant movement of the media has a self-cleaning effect on the filter media and prevents extensive bacteria growth. For cleaning the moving bed filter should be disconnected from the RAS and then backwashed approximately once per week.

The carrier media supports microbial biofilm growth by providing a large surface area. Typically, MBBR are filled 40-60% with biocarriers, creating an absolute surface area of 300-600 m2/m3 bioreactor volume. Air movement creates shear forces on the biofilms and keeps growth and breakdown of the biofilm in equilibrium. If the biofilm on the carriers gets too thick, then aeration is too low, and if it is non-existent, then aeration is too high. A major advantage of MBBR is the degassing and aeration by air flow, which is not provided by fixed bed filters.

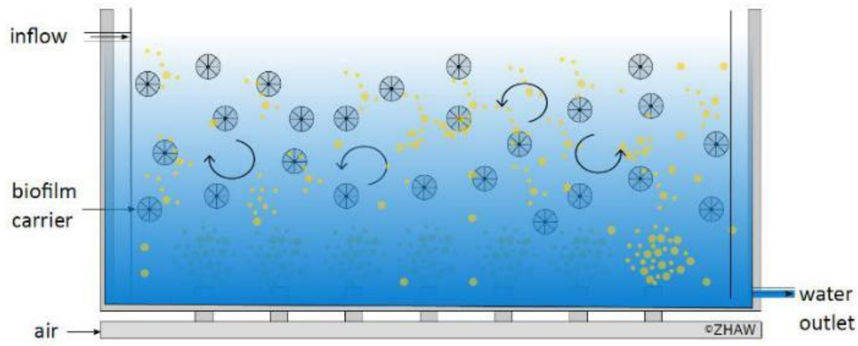


Figure 24: Basic construction of a moving bed biofilter reactor

Fixed bed filters have fixed biofilter media (Figure 25). The fixed bed filter also works as a solids removal device as it has filtration capabilities to filter out leftover solids and organic compounds that have not been filtered out in the solids separation unit. If the organic loading is higher than the natural degradation on the surface, the filter cake can become clogged by particles and bacteria growth. The filter needs to be backwashed regularly and the backwash water treated separately (by sedimentation etc.).

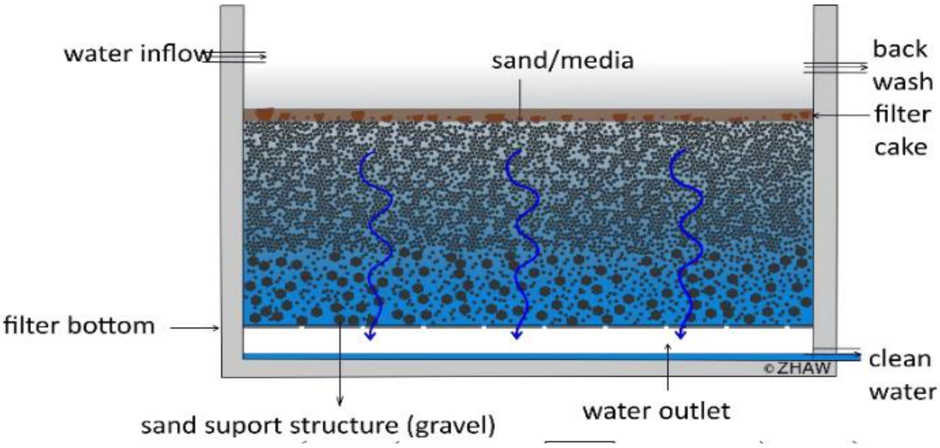


Figure 25: Basic construction of a fixed bed filter.

Trickling filters are the last of the three common filter types and work by trickling water through a pile of biofilm carriers (Figure 26). The biggest benefit of the trickling filter is the high degassing effect through the high water to air surface caused through the trickling. The main disadvantage are the high pumping costs needed to bring the water to the required height. ,. Since these carriers are not moved regularly like in a MBBR, the biofilm grows thicker on these carriers and reduces the nitrification rate. Trickling filters are very common in aquaponics, since they enable gas exchange (degassing of CO2 and aeration) in the one step. In addition, they only need water circulation and no additional aeration device like MBBR (e.g. a blower), which makes them a very easy to build system.

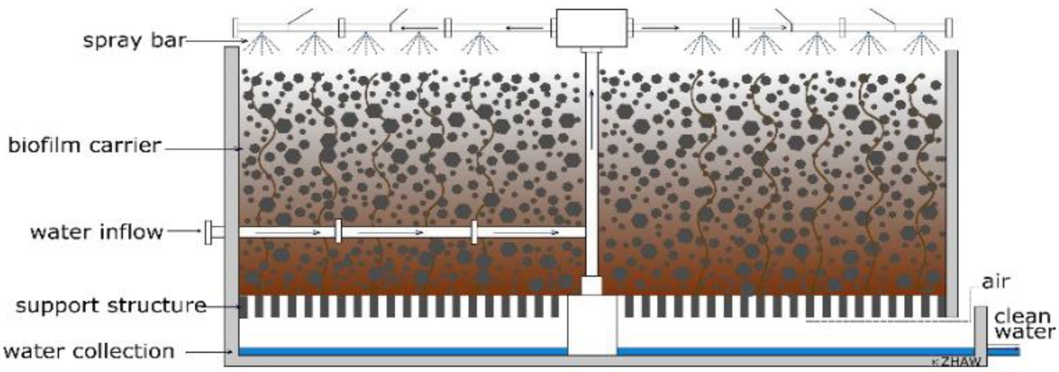


Figure 26: Basic construction of a trickling filter.

Biofilter type	Pros & Cons
Moving bed biofilm reactor (MBBR)	Nitrification ++ Filtration – Degassing +
Fixed bed filter	Nitrification + Filtration + Degassing -
Trickling filter	Nitrification + Filtration – Degassing ++ (if aerated)

Figure 27: Types of biofilters and their pros and cons in terms of system performance: moving bed biofilm reactor (MBBR), fixed bed filter and trickling filter.

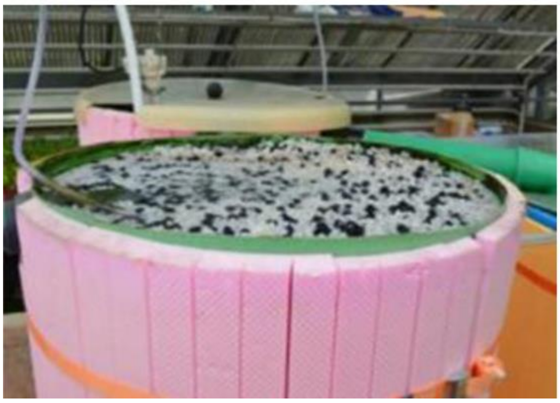



Figure 28: Two versions of suboptimal moving media biofilters: (left) biofilter containing too many biochips (right) biofilter with no aeration (photo: U. Strniša)

1.2.4.2.2 Aquaponics

In systems with low fish stocking density, a media growing bed can take over the role of both solids removal and biofiltration. If the solids load is too high, clogging and anaerobic areas can occur, which reduce the efficiency of biofiltration. Therefore, if the growing bed is to function as a biofilter, either a very low fish stocking or a separate solids removal device are recommended.

1.2.5 Degassing, aeration and oxygenation

1.2.5.1 General

The fish tank(s), biofilter and grow bed(s) all need appropriate aeration. There are many ways to provide this, including using airlift pumps, water sprays, paddlewheels, rotors, blowers, and compressors. As with water pumping, water aeration needs to be reliable and energy-efficient. Aeration in smaller systems can be provided by using an energy-efficient and long-lasting air pump and food-grade vinyl tubing connected to airstones placed at or near the bottom of the tanks and grow beds. Air pumps are generally not large enough for aerating larger systems, which tend to use a regenerative blower or an oxygen generator.

Gas transfer between the liquid and the gas phase occurs when there is sub-saturation in one phase. Gas solubility is dependent on pressure, temperature, salinity, and gas partial pressure. The transfer takes place over the contact surfaces between gas and liquid. **Aeration** increases the oxygen content in the water. **Degassing** removes gases such as carbon dioxide from the water.

1.2.5.2 Design

1.2.5.2.1 Degassing

Gases, especially carbon dioxide resulting from respiration of the fish and bacteria, accumulate in the system water. These can have harmful effects on the fish if concentrations become too high. Therefore, a degassing unit is usually added to intensive RAS. Gas outtake (degassing) is achieved by increasing the contact surface area between the water and the air, either by aeration of the water column, or by sprinkling water through the air. Different biofilters already have a high degassing effect: in a trickle filter the water passes through the air, while in a moving bed filter the air passes through the water. This may therefore make an additional degassing unit redundant.

1.2.5.2.2 Aeration

Dissolved oxygen (O_2) content is one of the most important water quality parameters in RAS and often the first constraint in emergency situations (e.g. in case of power cuts, pump failure etc.). There are numerous techniques to enrich dissolved oxygen in the water. Gas intake of water (aeration) can be enhanced by: (i) maximizing the oxygen/water contact area by using whirls or small bubbles; (ii) maximizing the oxygen/water contact period by using small bubble diameter and/or by a slow water flow; (iii) increasing the pressure (increases solubility) – water level, pressure vessel; and (iv) increasing partial pressure of O_2 (increases solubility) – pure oxygen.

In aquaponics, air pumps and air stones are used to force air into the water to provide plant roots and fish with oxygen. Air pumps are widely available in a range of sizes, from very small up to very large with a capacity to run from one to many airstones, each of which introduces hundreds of tiny bubbles of fresh, oxygen-rich air into the solution. While it is easier to push air out of an airstone that is in shallow water, you do not get as much oxygen into the water as you do if the airstone is deeper. When the airstone is deeper the large number of bubbles that come out are smaller because of the higher water pressure, which together have a greater surface area than fewer larger bubbles, and they have to travel further to the surface, with the surrounding water absorbing oxygen from the bubbles all the way to the top of the tank where they burst at the surface.

1.2.5.2.3 Oxygenation

In intensive RAS the oxygenation technologies depend on using pure oxygen rather than simple aeration which becomes impractical at certain fish densities. The oxygen is either produced on site with an oxygen generator, or supplied by an external firm and stored in high pressure tanks outside the aquaculture facility. Some strategies for increasing the oxygen supply in intensive fish farming include:

- Increasing freshwater supply
- Using the freshwater inlet as a water fall, spraying the water through the air
- Using air blowers to vent in air bubbles at the bottom of the tank (maximum depth is 2 m, otherwise N2 supersaturation occurs)
- Using pure oxygen from either pressure gas tubes (used for backup systems/transport only due to the high costs), liquid oxygen tanks (standard for RAS producing > 5 t/y), or oxygen generators (enrichment from ambient air up to 98% purity)
-

1.2.5.2.3.1 High efficiency oxygen input

The basic oxygenation technologies are the U-pipe, oxygenation cone, and low head oxygenator (Figure 29).

	U-Pipe	Cone	LHO
Principle	Increase in pressure by a water column; long water/oxygen contact distance	Germ reduction by DANN damaging	Excess pressure by water column, high contact surface of water/oxygen
Pressure Loss	No	High (2-3m, 0.2-0.3 bar)	Medium (ca. 1m, 0.1 bar)
Efficiency	High	High	Medium

Figure 29: Characteristics of different possibilities of high efficiency oxygen enrichment in RAS

One simple oxygenation technology to dissolve oxygen into the system water is the **U-pipe** (Figure 30). Oxygen is injected at the bottom of a 10-30 m deep pipe through which the system water flows. Due to the high hydraulic head, the high pressure leads to high dissolution of the oxygen into the water column. However, as this technique requires structures to be built deep into the ground, the method is often not implementable in practice.

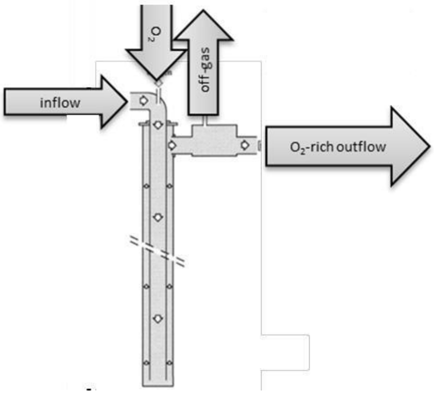


Figure 30: U-pipe

An **oxygenation cone** (Figure 31) uses the same principle as a U-pipe. The difference is that the high hydraulic pressure is induced by a pump (which uses a lot of energy). This technology is especially suited to cover peaks in oxygen demand, and it has a high efficiency in terms of oxygen dissolution

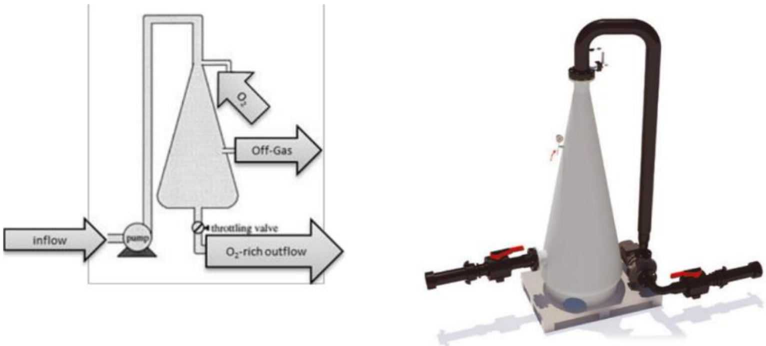


Figure 31: Oxygen cone for dissolving pure oxygen at high pressure

The **low head oxygenator (LHO)** (Figure 32) uses another method of oxygen enrichment. Water flows through a perforated plate and causes a high water to gas surface area in the mixing chamber below. LHOs operate very economically, although they cannot achieve oxygen concentrations as high as cones can.

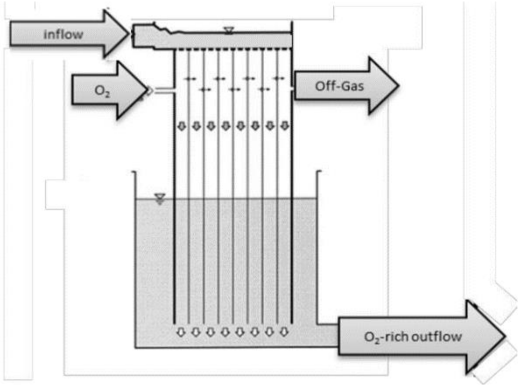


Figure 32: Low head oxygenator

1.2.5.2.3.2 Low Efficiency Oxygen Input

In extensive fish ponds low efficiency oxygen input is usually sufficient. This is achieved by (i) keeping the water cool, as this dissolves more oxygen, and (ii) increasing the water movement. Different modes of aeration can support this.

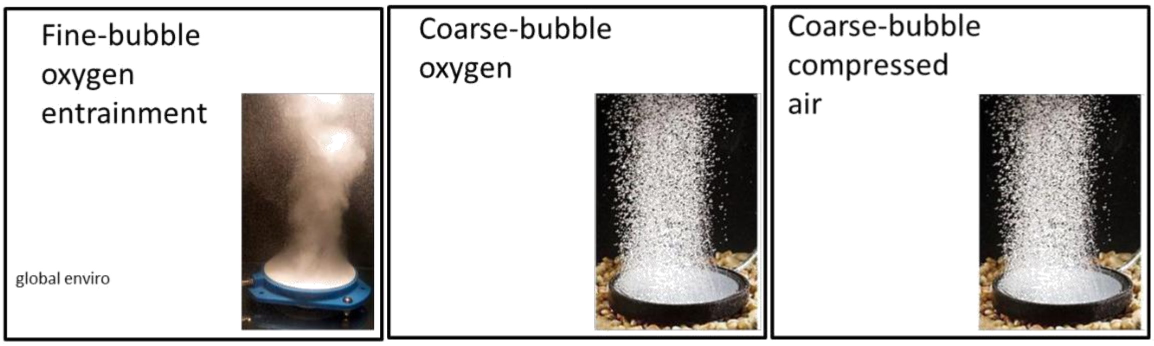


Figure 33: Different possibilities of low efficiency oxygen enrichment in aquaculture

	FINE-BUBBLE OXYGEN ENTRAINMENT	COARSE-BUBBLE OXYGEN	COARSE-BUBBLE COMPRESSED AIR
APPLICATION	Many fine bubbles slowly rise. Small bubbles have high surface to volume ratio	High concentration gradient (because it is pure oxygen). Most of the time used for emergency oxygenation	Does not need pure oxygen but has a low efficiency because only 21% is oxygen. The rest is N2 etc. Can lead to N2 over saturation
PRESSURE LOSS	1.5 bar	Beginning from 300mbar + water column	Beginning from 300mbar + water column
EFFICIENCY	Medium (up to 20%)	Low (5%)	Very low (1%)

Figure 34: Characteristics of different possibilities of low efficiency oxygen enrichment in RAS

1.2.6 Temperature control

1.2.6.1 General

Maintaining an optimal water temperature in the culture system is most important as the growth rate of coldblooded aquaculture animals is directly related to the water temperature.
In an indoor recirculation system the heat will slowly build up in the water, because energy in the form of heat is released from the fish metabolism and the bacterial activity in the biofilter. Heat from friction in the pumps and the use of other installations will also accumulate. High temperatures in the system are therefore often a problem in an intensive recirculation system. In cold climates heating of the water can be necessary (Bregnballe 2015).
In most types of aquaculture temperature cannot be controlled and depends upon the amount of solar radiation, air temperature, or the temperature of water passing through the culture unit (Boyd 2018). In indoor recirculation systems the water temperature can be controlled either by bringing in heated or cooled water indirectly through air temperature control, or directly by heating or cooling the water using gas, oil, electricity, or ventilation for the optimization of the aquaculture production. Heating/cooling costs can be lowered by recovering the energy by the use of a heat exchanger. Energy in the discharge water from the farm is transferred to the cold incoming intake water or vice versa. This is done by passing both streams into the heat exchanger where the warm outlet water will lose energy and heat up the cold intake water, without mixing the two streams. Also on the ventilation system a heat exchanger for air can be mounted utilizing energy from the out-going air and transferring it to the in-going air, thereby reducing the need for heating significantly (Bregnballe 2015).

1.2.6.2 Design

1.2.6.2.1 Heating

In cold climates heating of the water can be necessary. The heat can come from any source like an oil or gas boiler or a heat pump and is, independent of energy source, connected to a heat exchanger to heat the recirculated water. Heat pumps are an environmentally friendly heating solution, and can utilize energy for heating from the ocean, a river, a well or the air. It can even be used to transfer the energy from one recirculation system to another, and thereby heat one system and cool another. Usually it utilizes energy from e.g. the air using a heat exchanger, moves the energy to the recirculation system that is calling for heating and releases the heat through another heat exchanger (Bregnballe 2015).

1.2.6.2.2 Cooling

Using the intake water is a fairly simple way of regulating the temperature. By adjusting the daily intake amount of cool fresh water into the system, the temperature can be regulated (Bregnballe 2015). However, there are reasons to avoid this method, as it implies an increase in water demand and the

amount of discharge water to be treated. This negates one of the main benefits of a recirculation system, namely reusing the water supply as much as possible (Timmons & Ebeling, 2010).
In controlled environment rooms, a cooler can be used to blow cold air into the room and keep the temperature down.

1.2.7 Pumps and pumping pits

1.2.7.1 General

A pump is to RAS what the heart is to the human body. If it fails, then the result can be catastrophic. Therefore no expense should be spared when buying a pump. One can use speed controlled pumps to reduce the flow if needed. By using a series of pumps with check valves, the chances of system failure can be reduced. It is also a good idea to build in some redundancy by using two or three pumps in parallel. Before buying a pump, the pressure losses in the pipes should be calculated, for example with the help of this online calculator: http://www.pressure-drop.com/Online-Calculator.

There is a wide range of pumps on the market but they can be divided into two main categories: submersible pumps or inline (centrifugal) pumps. Submersible pumps are immersed in the tank water which helps to keep them cool. They are usually less efficient than inline pumps and are more suitable for smaller systems. Inline or centrifugal pumps are air-cooled pumps and are located outside the tank. They can have higher powered engines capable of pumping large quantities of water.

When sizing the pump for the aquaponic system, the flow rate has to be determined first –i.e. how much water the pump can move over a given time period. It is usually measured in litres per minute or litres per hour. The pump should be able to recirculate the entire volume of water in the system. This can vary from 3 times per hour in very intensive systems to only a few times per day in extensive systems. Generally, it is better to purchase a more powerful pump since it will allow for flow adjustments.

Normally the fish tank and growing bed will be at different levels. The greater the distance or the larger the head, the more energy is required to pump the water. Anything that can be done to minimize the head will make the entire system more efficient. Generally, most pumps come with a chart that combines flow rate and head height. If not, then usually the maximum flow rate (Qmax) and maximum pumping height (Hmax) are stated. Pumps have their optimal pumping efficiency at Hmax/d, which is normally around Qmax/2.

The cost of energy used to run the pump is an important part of the cost structure for running an aquaponic system. It is therefore important to know the electrical consumption of the pump you plan to purchase, which means knowing the number of watts the pump uses. The ideal pump will get the job done while using the smallest amount of energy possible. When purchasing a pump it's also important to purchase a backup pump in case the first one breaks down, or operate the system with two pumps in parallel (highly recommended) and have one backup pump.

1.2.7.2 Design

If it's needed to recirculate 10m^3/h for 2m, then first decide to use one or two pumps. Using two pumps in parallel, each pump has to pump 5m^3/h for 2m including friction losses in the pumping pipe. So, each pump is with Hmax = 4m and Qmax = 10m^3.

1.2.8 Waste water treatment

Wastewater treatment is an important cost factor of intensive RAS. This is normally charged per m³ of wastewater that leaves the facility and enters the public wastewater plant. To minimize the volume of wastewater it is feasible to treat the sludge water that results from the solids separation. In this way even a low-tech filtration system can achieve a significant reduction of the final wastewater volume.

Fish sludge is rich in nutrients that can be reused as fertilizer. There are several alternatives to dumping it into the sewage system, including the following:

- storing and re-using it in traditional gardening and agriculture; however, this may be prohibited by law
- co-composting with structurally rich green waste (tree cuttings, straw)
- vermicomposting (composting process using various species of earthworm).
- anaerobic digestion and reintroduction of digestate into the aquaponic system (Goddek et al. 2016).
- denitrification to shift the N:P ratio in the aquaponic system in order to reduce P limitation.

Figure 35:(left) sludge storage tank (photo: U.Strniša); (right) compost (photo: pixabay)

1.2.9 Ventilation

Many RAS designers make the mistake of ignoring the ventilation requirements associated with a building that houses an RAS. Ventilation requirements for controlling environmental conditions of humidity, temperature, and $C0_2$ should be approached in the same way that a ventilation balance is done for chickens or cows to maintain an acceptable air quality environment. For an RAS, we have fish instead of these warm blooded animals, the controlling environmental conditions are typically humidity and then $C0_2$.

1.3 Management of recirculating aquaculture system (RAS)

1.3.1 Stocking density

Stocking density is a very important factor that has to be decided in advance when designing a RAS. Stocking density can be defined in different ways (Figure 36), and it is important to be aware when and why different definitions are being used.

	DENSITY OF INDIVIDUALS	BIOMASS DENSITY
DENSITY PER SURFACE	(#/m^2) Independent of tank depth Relevant for bottom-dwelling fish	(kg/m^2) Independent of tank depth Relevant for bottom-dwelling fish. It is often higher than for bigger fish than for smaller species
DENSITY PER VOLUME	(#/m^3) Is often high for small fishes even though the biomass density is higher	(kg/m^3) Relevant for free swimming species

Figure 36: Stocking density definitions

Low and high stocking densities in aquaculture systems have several consequences for the management of a RAS (Figure 37).

INFLUENCING FACTORS FOR SYSTEMS WITH THE SAME ANNUAL PRODUCTION	HIGH DENSITY	LOW DENSITY
CHANGE WATER PARAMETER	Fast change	Slow change
RESPONSE TIME (FOR EXAMPLE TO PUMP FAILURE)	Is shorter. More stress for the fishes	Is longer. The system operation is safer
CAPACITY OF THE FISH TANKS FOR A GIVEN PRODUCTION VOLUME	Less capacity for the same production volume	Higher capacity needed. This can be compensated partly by using deeper basins. However, these are more expensive and need a more expensive pipe and pumping system
NECESSARY CIRCULATION/DISPLACEMENT RATE FOR A GIVEN PRODUCTION (M^3/H)	Same	Same. Due to the slowness of the system, there are softer peaks = smaller components = less expensive hardware for water reconditioning
DISPLACEMENT VOLUME RELATIVE TO TANK VOLUME	High	Low
TANK DIMENSIONS	Smaller tanks with a high density of individuals are, depending on the species, more prone to stress	In larger tanks, easily scared fishes have a longer escape distance

Figure 37: Characteristics of low and high stocking density systems

1.3.2 Monitoring

Monitoring procedures should be defined according to the steps outlined in Figure 38. RAS or aquaponic systems are complicated, and consist of many parts. Many things can go wrong, so the operators have to remain permanently alert.

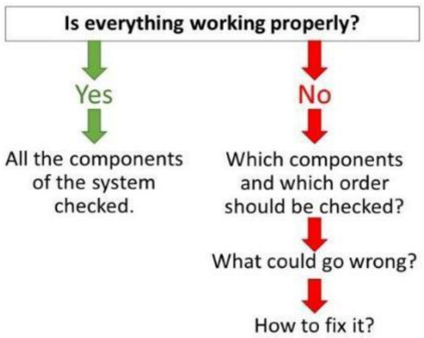


Figure 38: The logical steps of devising a monitoring procedure

The top priority of system management is the health of the fish and plants. Therefore, monitoring should be prioritised according to the 'life support priorities' (Figure 40). Table 6 lists important items that should be monitored on a daily basis.

TYPE / SYSTEM	CAUSES
BEYOND YOUR CONTROL	Floods, tornadoes, hurricanes, wind, snow, ice, storms, electrical outages, vandalism/theft
STAFF ERRORS	Operator errors, overlooked maintenance causing failure of backup systems or systems components, alarms deactivated
TANK WATER LEVEL	Drain valve left open, standpipe fallen or removed, leak in system, broken drain line, overflowing tank
WATER FLOW	Valve shut or opened too far, pump failure, loss of suction head, intake screen blocked, pipe blocked, return pipe ruptures/breaks/glue failures
WATER QUALITY	Low dissolved oxygen, high CO2, supersaturated water supply, high or low temperature, high ammonia, nitrite or nitrate, low alkalinity
FILTERS	Channelling/clogged filters, excessive head loss
AERATION SYSTEM	Blower motor overheating because of excessive back pressure, drive belt loose or broken, diffusers blocked or disconnected, leaks in supply lines

Figure 39: What can go wrong?

PRIORITY	PARAMETER	RESPONSE TIME
HIGH	- Electrical power - Water level - Dissolved oxygen	Very fast (minutes) Alarm needed!
MEDIUM	- Temperature - Carbon dioxide - pH	Moderate response time (hours)
LOW	- Nitrogen forms (ammonia, nitrite, nitrate) - TSS	Slowly changing parameters (daily or weekly monitoring)

Figure 40: Prioritisation of monitoring and response

Electrical power	Single and three phase supply, individual systems on life-saving GFCI outlets
Water level	Culture tank (high/low), supply sumps to pumps (high/low), filters (high/low)
Aeration system	Air oxygen pressure (high/low)
Water flow	Pumps, culture tanks, submerged filters, in-line heaters
Temperature	Air oxygen pressure (high/low)
Security	Pumps, culture tanks, submerged filters, in-line heaters

Figure 41 Important items that should be monitored daily

1.3.2.1 Some advice for system design and safety

- Choose sensors carefully, label everything, and include expansion capability in all components
- Aquaculture facilities are included under the National Electric Code: it may not be of concern to you, but it is to your insurance agent
- Install the sensors and equipment where they are visible and easily accessible for servicing and calibration
- Remember that water and electricity make for a fatal combination, so use low voltages (5 VDC, 12 VDC or 24 VDC or AC) to protect yourself and the fish
- Clearly label the sensor's armed and unarmed modes, preferably with LEDs at each station to show sensor status.
-

1.3.2.2 Some advice for system maintenance

- Have a well prepared maintenance manual accessible for staff to read
- Maintain a weekly/monthly/yearly maintenance scheduling plan and keep files of major service records and equipment manuals
- Maintain daily/weekly/monthly instrument check lists
- Perform regular (and some unannounced) system checks, including triggering each sensor and checking the operation of the automatic backup systems and phone dialer
- Provide staff training on handling routine alarms
- Ensure that staff are familiar with the complete operating system, including water supply, aeration, and emergency backup systems.

1.3.2.3 When to monitor water quality?

Fish digest according to the time they are fed, and the amount of faeces depends on the amount of ingested feed. Thus, the highest levels of ammonium are to be expected after the last feed (in the evening) and the lowest value before the first feed (in the morning). Therefore, measurements of water quality have to be done at the end of the feed in order to catch ammonia peaks (Figure 42).

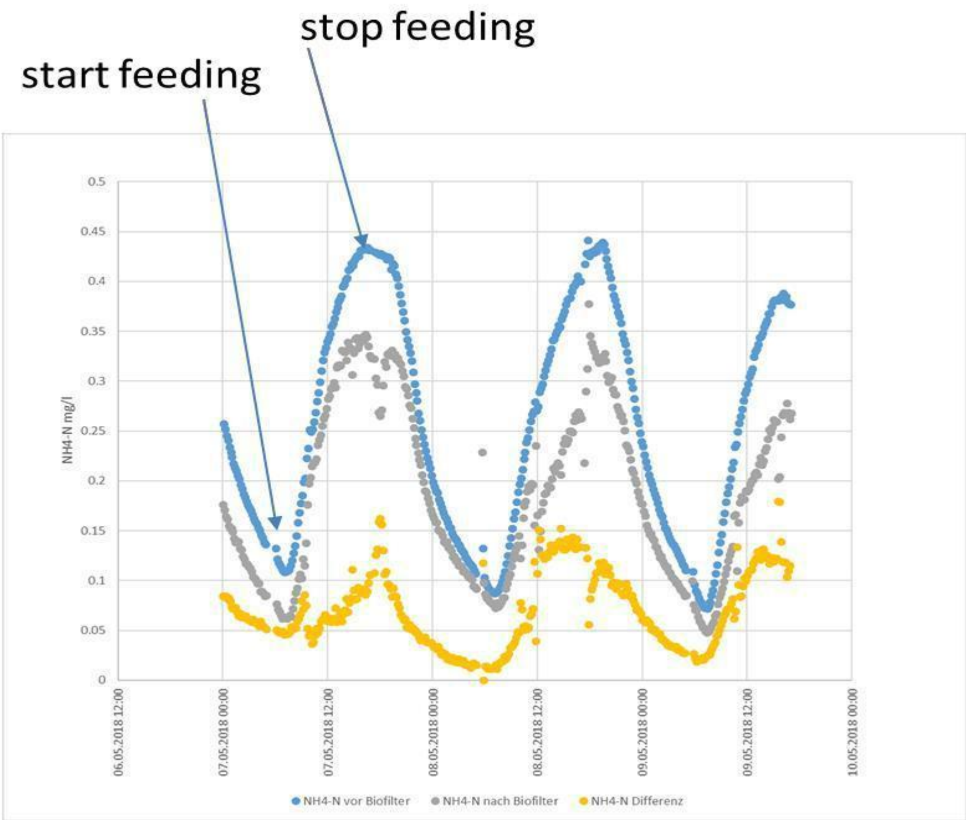


Figure 42: Daily time course of NH4-N concentrations in RAS water. Blue = before biofilter; grey = after biofilter; yellow = difference between blue and grey

Automated monitoring is becoming increasingly affordable. There are several data acquisition and control systems commercially available for applications in RAS and/or aquaponics. A monitoring system includes (i) sensors to measure the desired variables, (ii) an interface to convert the electrical information into a form readable by a computer or microprocessor, (iii) a computer, (iv) software to run the system, and (v) displays. It is important to match the components, in order for the monitoring system to work.

One of the most important functions of a monitoring system is to provide alerts to the system operator in the event of malfunctions and problems. If critical variables are sensed to be outside of acceptable limits, alarms need to be sent out. It is important to design and test the monitoring and alarm system so that false alerts are not sent out too often. Too frequent false alarms make it less likely that the operator(s) will respond (Timmons et al. 1999). Alarms must be constructed an operated so that pertinent individuals are alerted. Visual and audible alarms can be placed in key areas within a facility to alert workers of problems. Outside normal working hours remote alarms (usually via SMS messages) need to be employed.

1.4 Planning the recirculating aquaculture part for an aquaponic system

In aquaponics, it is very important that the input and output of nutrients is in balance over the entire plant growing period. This balance can mainly be controlled using two different approaches:

- Approach 1: An existing recirculating aquaculture system (RAS) is used to dimension the corresponding hydroponic unit with plants (Figure 43).
- Approach 2: The RAS is dimensioned based on the desired plant and fish production (Figure 44).

The aim of dimensioning the RAS part of an aquaponic system is to adjust the different water treatment stages in order to achieve both good water quality for the fish, and sufficient nutrient supply for the plants. It is always an advantage if the system is as unaffected as possible by seasonal fluctuations (temperature, dissolved oxygen, ammonium, nitrite and nitrate). In general, it can be said that a large water volume and low stocking densities make systems more stable. It is important that the whole year is planned and that differences in the fish and plant species as well as the growth stages of all species are taken into account. As support for this planning, it is recommended that the 'Planning Basis for Dimensioning the Recirculating Aquaculture Part of an Aquaponic system model' is used (Tschudi 2018).

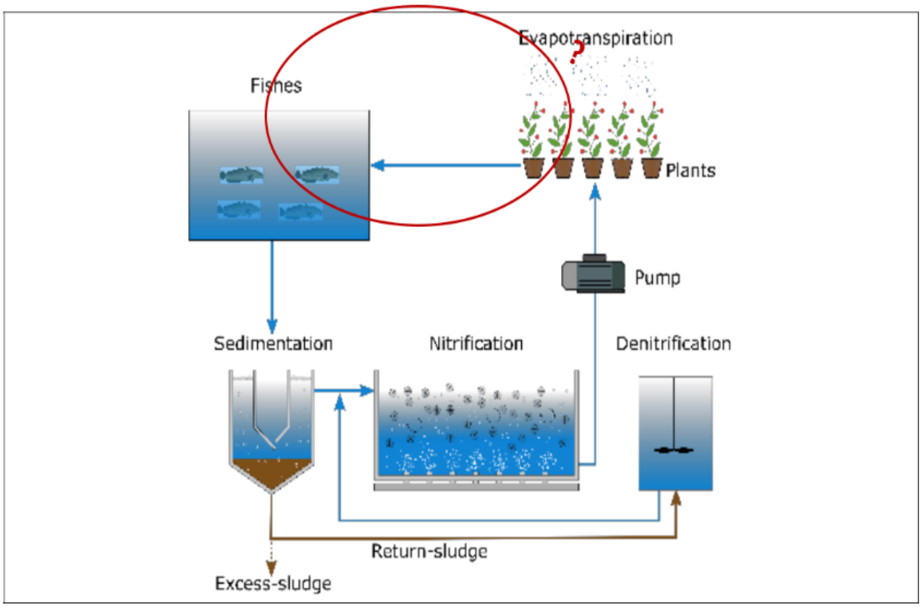


Figure 43: Dimensioning of the plant nutrient uptake based on existing RAS dimensions

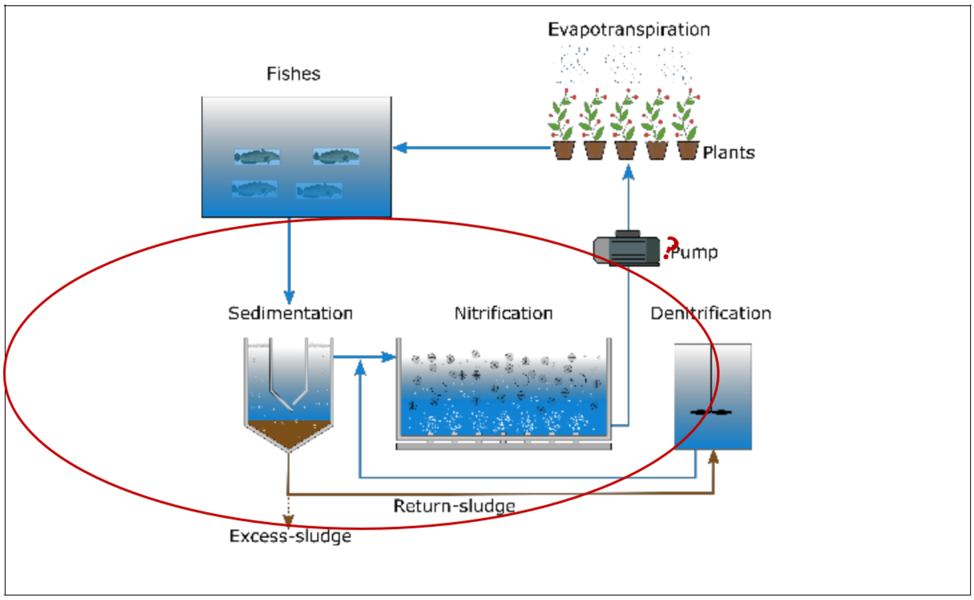


Figure 44: Desired plant and fish production and corresponding dimensioning of the RAS

1.5 Fish nutrition

1.5.1 Introduction

Feeding and fish nutrition are fundamental aspects of aquaculture, both in terms of fish growth and in economic terms. Proper feeding depends on the development of quality feeds and on choosing appropriate methods to distribute the feed to the fish in the tanks. Apart from affecting growth, feeding can also affect fish health and welfare, which depends in turn on how much we know about the requirements of each species. Each species has its own natural history and well defined stages of growth, which should be understood in order to provide optimal care.

The candidate fish species for aquaponics occupy well defined ecological niches in their natural habitat. For that reason we need to provide adequate conditions for proper development, including housing conditions, which means defining the correct temperature, salinity, water quality, and speed of water flow. Normally the most demanding phases are the maintenance of breeders and the fertilization/incubation of ova or eggs, but aquaponic production will normally be dealing with later stages, usually called ‘on-growing’. As the scale of aquaculture and aquaponic farms increases, it becomes more complex to maintain a large number of production phases in the same installation, so companies become specialized in one or two stages, such as breeding or on-growing. In the case of aquaponics, where fish are maintained in recirculating aquaculture systems (RAS), we normally use juveniles which are grown to adults, aiming to simplify the fish production part of the system with only one or two phases, if possible.

In general terms, feeding in aquaculture differs in some fundamental aspects compared to terrestrial mammals. Livestock on land normally self-feed using what are known as ad libitum feeders (each animal can choose when to approach the feeder and how much to eat at any given time of the day). In that case it is relatively easy for the farmer to detect the ration that was really ingested. In the case of aquaculture and aquaponics, fish can also use self-feeders but it is much more difficult to judge how much feed they actually consume. The danger is that any extra feed that falls into the water and is not ingested becomes waste that 'pollutes' the system. Efforts need to be made, therefore, to estimate the feed to be distributed and the precise ration that the fish need.

One way to distribute the feed is by hand from outside the tanks, spread over the whole surface area of the water, observing the behaviour of the fish until they seem to be satiated, and then feeding is stopped. Since the fish are feeding underwater, it is not that easy to know when they stop feeding or how much they ate, or even if some fish ate more than others. The more we know about a species, the more we know about their feeding habits. For example, Nile tilapia in the wild are omnivorous when young (juveniles), eating both zooplankton and phytoplankton, while they become more herbivorous as they get older (< 6 cm long) (FAO 2018). Trout, on the other hand, are mostly carnivorous throughout their lives, with a diet almost exclusively based on insects and any smaller fish they can manage to catch. In any case, the perception and knowledge of the people who are in charge of feeding is very important, especially if feeding is done manually. For more information on the feeding habits of different species, see the Aquaculture Feed and Fertilizer Resources Information System, run by the Food and Agriculture Organization of the United Nations (FAO 2018).

Another way is to use automatic feeders instead of manual feeding. Here we might depend on technological developments such as underwater cameras to detect when the fish are no longer eating. All the feed that goes into the tank becomes a part of the system, whether it is eaten or not. Indeed, fish feed is the main external element of any aquaponic system and should be carefully controlled. Non-ingested feed remains in the tank and causes two problems, one associated with its cost and another associated with its elimination. These two problems underlie the need for adequate designs.

The hydraulics of the system should facilitate the removal of the uneaten feed. Normally this involves tapering the tanks so that the bottom part is narrower than the top, and promoting a swirling motion or current so that faeces settle on the bottom and can be removed efficiently. If the design is deficient, cleaning will be more complex and the fish may be bothered by the frequency of maintenance routines. Any decrease in the sanitary conditions of the tanks will have immediate consequences on the welfare of the fish, and on the profitability of the farm. So, even if we know the nutritional needs of the species, a poorly designed installation will make it difficult to provide adequate requirements for good fish welfare, and feed will be wasted.

1.5.2 Energy requirements

As with all living animals, fish require energy, and that energy is provided by the oxidation of the organic components in feed. Fish require energy to carry out their daily activities, such as breathing and swimming, and to transform, restore, and grow their body tissues. The energy requirements of fish depend on their physiological state and on the environmental conditions. In general fish make a more efficient use of the energy ingested compared to terrestrial mammals, due to the following reasons:

1- Aquatic species are poikilotherms, which means that their body temperature is the same as the surrounding water, so they do not need to spend energy heating up their body or keeping it at a constant temperature, as occurs with terrestrial livestock;
2- Since they live in water, fish do not require a strong body skeleton to support their weight under the full pressure of gravity, as in terrestrial livestock, nor do they require the costly metabolic processes required to maintain that skeleton;
3- Nitrogenous waste in fish is eliminated as ammonia directly from the gills which consumes less energy than having to make urea or uric acid and then eliminate it, as is done by mammals and birds.

Figure 45 provides an overview of the balance of nutrients and energy in fish. If we assume that it has ingested all the feed provided, the energy is distributed percentage-wise among different physiological processes, within ranges. If maintained under stressful conditions (poor lighting, low water quality, inadequate stocking densities), where the fish are alive but not comfortable, about 40% of the feed energy will be consumed just to cope with the stress, leaving only 30% for growth. On the other hand, under optimal conditions, fish will use up to 40% for growth. Obviously, the economic viability of an aquaponic system will depend on the optimal use of the energy provided. To do that we have to ensure that they ingest all of the feed, and that we provide optimal housing conditions so that the fish are not overly stressed.

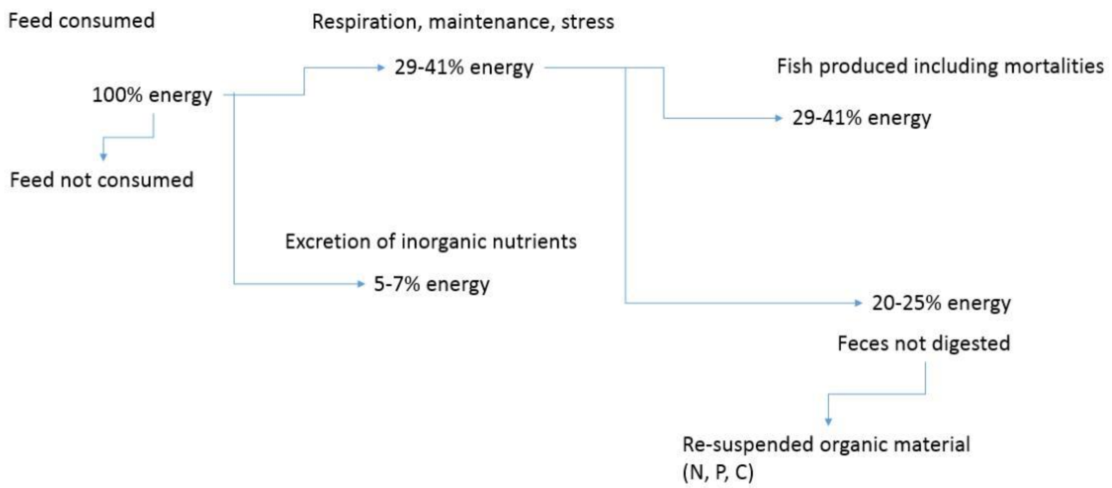


Figure 45: Balance of nutrients and energy for fish kept in recirculating systems

1.5.3 Main interactions between ingestion and environmental factors

As commented above, we should be able to house each species according to its requirements. For that we first need a profound knowledge of the species that we are going to work with before we begin to grow the fish or start the installation. Once we have this information, we should be able to maintain the adequate housing conditions in our system, which in this case is related to aquaponic systems.

1.5.3.1 Abiotic factors

The main environmental aspects to consider and that have a direct effect on production are the following:

1. Physico-chemical parameters of the source water, which are independent of the aquaculture activity itself:

    a. Water temperature, which regulates all metabolic processes

    b. Water salinity or conductivity

    c. Turbidity and total suspended solids

    d. Any potentially toxic compounds in the source water. The initial quality of the water is one of the basic success factors in the installation

2. Physico-chemical parameters of the tank water:

    a. Dissolved gases: fundamentally oxygen, which should be monitored continuously and is required by fish for normal function. In parallel, carbon dioxide is produced by fish respiration, and other gases are present in the circuit, such as nitrogen (that can appear during the over-saturation of pumped water), or hydrogen sulphide or methane from the anaerobic decomposition of sediments

    b. Dissolved micro- or macronutrients, which are related to the feed, including several elements vital for the development of the fish, such as phosphorus, iron, and especially the nitrogenated substances excreted by the fish

1.5.3.2 Biotic factors

Different species of fish are extraordinarily diverse in terms of their social requirements, such as stocking density. Historically, fish chosen for aquaculture are robust under different conditions, which makes it easier to choose adequate management. That includes carrying out daily tasks on the farm without generating many sanitary complications in the fish. This is also the case for aquaponics, where the most popular fish is tilapia, well known for its hardiness.

However, in the beginning, we first had to domesticate wild species, which were normally difficult to manage, reproduce, and grow, but had a high economic value. That high value covered the costs of production of delicate species. A clear example is rainbow trout, which in the beginning was a very complex species, hard to produce and manage, even though now it seems relatively simple. Any poor management and inadequate movement of the fish produced stress and even loss of scales, which led to infections that brought on or facilitated disease and other common problems of fish that are stressed. Examples of species that are currently being domesticated, and have not reached their full potential in aquaculture, are burbot (*Lota lota*) and grayling (*Thymallus thymallus*). Technological development and accumulated knowledge have drastically improved the techniques used in the routine operations on farms, such as sampling of the fish, counting the fish, movement of live fish, etc. The main aspects that will influence the welfare of the fish in the tanks include:

a. Social structure:

Depending on the species, some are quite territorial, and we must manage these characteristics in the tanks. For example, we know that trout are quite territorial, and that they require frequent size classification during the initial phases of growth in order to avoid the appearance of dominant fish that will damage the smaller fish. In that case it is better to keep the fish within a narrow size range in separate tanks in order to improve production. We also know that tilapia and Clarias species show two different modes of behaviour: territorial if at low densities, and swarming/schooling if at high densities. Thus, low densities are not always better for all fish species.

b. Fish density:

Each species has a minimum and maximum stocking density below or above which problems may arise and fish welfare will be jeopardized. Density is normally measured in kg/m3 and varies depending on the system. Some high output industrial RAS systems grow tilapia above 60 kg/m3 but normally aquaponic systems use lower densities, around 20 kg/m3 (see for example the Aquaponic Gardening Rules of Thumb), although values can range widely depending on fish size and RAS system.

c. Human disturbance:

This depends on the species. Tench (*Tinca tinca*), for example, are quite flighty, and can hurt themselves by bumping into the tank walls when disturbed or even when they notice human shadows. One solution is to put curtains around the tanks to avoid being seen, or to set tanks on rubber supports to minimize vibrations from human steps or machines.

d. Prey or feed:

The size of the feed should be appropriate for the size of the fish, and distributed throughout the tank so as not to promote dominant fish. Otherwise less proactive fish will not gain weight and tanks will need to be size sorted more often, which is stressful.

e. Predators:

The presence of predators, such as cats, dogs or birds close to the tanks, can stress the fish a lot, and contact needs to be avoided by using artificial boundaries such as fences.

f. Noise:

Loud noises, such as music (especially a strong bass sound) can be stressful for fish as well.

1.5.4 Proximate composition of fish feeds and essential nutrients

When research began on fish feeds more than 50 years ago, scientists first analysed the natural diets of the species in question. Trout, as an example of a carnivorous fish, had a natural diet that consisted of 50% protein, 15% fat, 8% fibre, and 10% ash, which is high in protein compared to terrestrial mammals. Ever since then researchers have been trying to find the right balance of protein, carbohydrates, fats, fibre, vitamins and minerals for fish used in aquaculture (Bhilave et al. 2014).

One of the most important components of any fish feed is protein. All proteins are composed of amino acids in different proportions. Thus, modern nutritionists tend to look at protein requirements in terms of amino acid requirements and aim to identify the ideal levels of the most important ones. This makes the whole system more efficient since fish are not getting any extra amino acids (that are then wasted), and have enough of the essential amino acids to grow healthily. Usually the level of protein is the first and most important question to ask when designing a diet. This is also a key issue in aquaponics since the protein in feed is the source of all the nitrogen waste that will later be used by plants.

Carbohydrates are composed of glucose, the main energy source for animals. In fish feed the most commonly found carbohydrate is starch, which helps to hold feed pellets together and provides an inexpensive source of energy. Although typically found in low amounts in fish feed, recent developments have led to an increase in its use. Now, in an effort to spare protein, that is, to reduce the amount of amino acids that are broken down to make energy, fish nutritionists are supplying more carbohydrates, with the advantage that the latter are also cheaper than protein (e.g., Lazzarotto et al. 2018). The only drawback is that this approach effectively makes many carnivorous fish more herbivorous, or vegetarian, since the extra carbohydrates are mostly of plant origin. Many studies in the past 5 years have been analysing how this can affect fish growth and welfare, and the results are promising.

Fats are made up of triglycerides or fatty acids which, like carbohydrates, provide energy to fish and, unlike carbohydrates, can be stored in different organs. Many fish, especially from colder waters, rely on high levels of fat in their diet (less than 15%), including omega-3 and omega-6 fatty acids. Fatty acids are also needed to transport fat-soluble vitamins. The relatively high levels of fat in most fish diets means that anti-oxidants are required to maintain their stability, avoiding degradation during processing and storage of the feed (Harper & Wolf 2009).

Crude fibre is the indigestible or difficult to digest part of feed that helps to promote gut motility (peristalsis). Ash represents the minerals in feed, such as potassium, phosphorus, copper and zinc. Exceeding the minerals that can be assimilated by the fish means that the extra minerals will be dissolved in the water. This is also important in aquaponics since we can design feeds that provide excess minerals that will end up being excreted by the fish and will therefore be available for the plants. However, it is usually a good idea to optimize feed for fish first.

An important concept in fish nutrition is the digestible protein to digestible energy ratio, often abbreviated as DP/DE. If the diet given to the fish is healthy and balanced, they will stop eating when they 'feel' their energy budget is reached. Energy can come from fat, carbohydrates or protein. As seen above, the most accessible source of energy is carbohydrate, followed by fat, and lastly protein. If the diet is high in protein compared to easily accessible energy (a high DP/DE), fish will have to eat more protein than they need to grow. Thus, that extra protein will not turn into muscle but will be broken down and used for other metabolic purposes, or simply wasted. On the other hand, if the DP/DE is low, then the fish will stop eating before then have enough to grow properly, and will be debilitated (Oliva-Teles 2012).

In summary, Figure 46 provides the general composition of a diet for adult trout (carnivore) and adult tilapia (herbivore), the latter being the most commonly used fish in aquaponics. The amount of vitamins and minerals is low compared to the other main components, and depends on the vitamin/mineral mix used by the feed producer. For example, the aquaponic system at the Arizona State University that is used to grow tilapia uses feed with 5mg/kg of folic acid and 66 mg/kg of vitamin E in terms of vitamins, and 7 mg/kg of phosphorus and 0.5 mg/kg of magnesium in terms of minerals (see Fitzimmons 2018), among others.

	TROUT[1]	TILAPIA[2]
PROTEIN	50	30
CARBOHYDRATES	17	46
FAT	15	9
FIBRES	8	5

Figure 46: Summary of feed composition (as percentage of dry weight) for a carnivore (trout) and a herbivore (tilapia). The remaining 10% includes ash with vitamins and minerals

[1]FAO 2018; [2]Tran-Ngoc et al. 2016

1.5.5 Types of feeds

In Europe, intensive aquaculture began at the end of the 19th century, when governments decided to breed fish to obtain fingerlings which were used to restock lakes and rivers (Polanco & Bjorndal 2018). Those fish represented an important source of protein for river communities, and helped to alleviate hunger. Efforts were made to promote the most appreciated species, such as salmonids, which are carnivorous. As production increased and fish were kept under intensive care for longer periods, farmers began to formulate feeds. In the beginning they captured macroinvertebrates in nearby water bodies, but that was seasonal and in limited supply. Later, fish were fed using waste products from slaughterhouses, which were chopped up into small pieces and thrown in the water directly. As a result, many salmon farms were established close to slaughterhouses.

Fish farms near ports used discarded fish from the fisheries but the supply was not always constant and was more difficult to organize as production increased. So farmers began making a paste with discarded fish that was blended together to make fish meal, to which they sometimes added plant protein. The paste could also be shaped into pellets, which facilitated spreading over many tanks, but since it was quite humid it could not be kept for very long periods before going bad. As time went on, fish nutritionists started to develop granulated feeds around the middle of 20th century. They were drier and were easier to formulate to the nutritional requirements of each species, and were much easier and cheaper to store.

Those first granulated or compound dry feeds facilitated the expansion of fish farms. Since then there has been intense research on the most appropriate and economically profitable raw materials to use in feed formulas. The whole process was improved by introducing the technique of extrusion, which applies high pressure to the feed paste during short intervals, increasing the temperature, making the granule lighter (allowing it to float in the water for longer periods) and allowing the incorporation of more fish oil. It also improved the compactness of the granules so that they did not dissolve immediately upon contact with water.

More recently efforts have been made to produce feeds that are more sustainable and organic. As mentioned above, for carnivores that means reducing the amount of fish meal in fish feed (and replacing it with plant protein like soya meal) and fish oil. For tilapia is also means reducing or eliminating any fish meal or fish oil, while maintain flesh quality. Recent research has focused on alternative protein sources for many types of fish, including the use of algae or insect meal.

1.5.6 Feeding strategies

Apart from using adequate feeds, we need to ensure that the pellets provided are the right size for the mouth of the fish. For small fish this usually means a fine powder and for larger fish a round pellet that can be several mm in diameter. For example, Aquaponics USA suggests using powder for tilapia from hatching to 3 weeks old, and then a fingerling crumble (1/32 inch or 0.9 mm) until they grow to about 2 cm in length, fingerling pellet (1/16 inch or 1.6 mm) until about 4cm in length, and grow out pellet (3/16 inch or 4.8 mm) after about 6 cm in length.

It is also necessary to distribute the feed adequately. Normally feed is thrown onto the surface of the tank and personnel perceive how the fish react – whether they move to the surface and begin to eat (generally a good sign), or whether they remain on the bottom of the tank (generally a bad sign). However, in neither case is it obvious whether they are eating properly, how much ends up in their mouths, and how much is wasted. Due to these problems it is quite easy to overfeed.

In general, feed is distributed to the fish according to feeding tables that are prepared by the feed producer in terms of water temperature and growth stage. But the perception of the feeder, the personnel giving out the food, is very important since he/she can tell how hungry the fish are, and that is related to health and welfare. More and more efforts are being made to automate the process, and systems have improved considerably, but we cannot underestimate the importance of observing the fish, which is probably the best and most direct method of understanding their status. While much research has been performed to optimize feeding for maximum growth, it is obvious that if we provide less feed than they need, they will grow less, and the producer will lose money.

In order to understand the feeding process we need to define some concepts, based on Figure 2, which was developed by Skretting, an important feed company. We need to define the concept of maximum ration, which is the theoretically ideal ration to be given to the fish. However, it is specific to each farm since it depends on external conditions such as water quality and temperature, as well as tank design. The main concepts and indices used commercially include the following:

1.5.6.1.1 Feed Conversion rate

This is the ratio between the amount of feed ingested (in grams) divided by the live weight increase. On a commercial level we sometimes use an 'industrial FCR' which is an approximate figure based on all the feed provided over a period of time divided by the tonnes of fish produced during that same period. In that case, if there was mortality, we do not subtract the feed consumed by the fish before their death. This industrial FCR provides an idea of the real production costs. Another similar index is the biological conversion factor (BCF), which is the kg of feed really consumed by the fish divided by kg gained. It is harder to calculate the BCF at an industrial level since the fish have to be handled and the feed put down their throats, but is useful when we want to know the maximum efficiency of newly developed feeds. FCR describes the amount of feed needed for one kg weight gain by the fish:

$$FCR = \frac{Food\ fed\ to\ the\ fish(kg)}{Liveweight\ gain\ (kg)}$$

This ratio reflects the nutritional and economic value of a feed. An FCR of 1 means that you have a live weight gain of 1 kg if you feed 1 kg of feed. The higher the FCR is, the higher your feed expenses are. Young fishes have a lower FCR (between 0.4 – 0.8), while adult fishes have an FCR between 0.9 – 2. The FCR depends on fish species and feed manufacturer. Sometimes you get more economic value with high quality food and the related better growth of the fish, in comparison to cheaper feed with a lower FCR.

1.5.6.1.2 Specific growth rate (SGR)

This represents the percentage daily growth of fish. It is specific for each species and related to fish size and water temperature. Like the FCR it is dimensionless (no units) and is useful for comparing data between farms or species. The SGR shows the daily average growth of a fish in percentage of its bodyweight:

$$SGR\ \left[\frac{\%}{d}\right] = \left(\frac{lnW2 - lnW1}{T2 - T1}\right) * 100$$

where W1 and W2 denote the weight of fish at the beginning and at the end of the growth period, respectively, and (T2-T1) denote the duration of growth period in days.

1.5.6.1.3 The daily feed rate (DFR)

The percentage of feed provided expressed as a percentage of fish weight (% fish weight per day). Normally this percentage is higher for younger fish (around 10%) and lower for older fish (around 1-2%).

1.5.6.1.4 Ration consumed

The ration really consumed by the fish.

1.5.6.1.5 Maintenance ration

The precise ration needed in order to maintain the fish at a constant weight without growth.

1.5.6.1.6 Maximum ration

The ration needed in order to obtain the maximum possible growth.

In Figure 47 we can visualize the concept of the maximum ration, which provides maximum growth of the species under culture. This maximum ration will be specific to each farm, and depends on local conditions. As we get nearer to the maximum ration, growth will increase, but if we go over the limit, we are wasting feed. However, in general terms it is advisable to feed small fish more than the maximum ration, since the waste will be small due to the small existing biomass, and we will tend to maximize growth. But in the case of final growth, we tend to be more prudent, since there is a large biomass in the water, and any extra feed that is lost will be costly and will increase the negative environmental impact, making it necessary to clean it up.

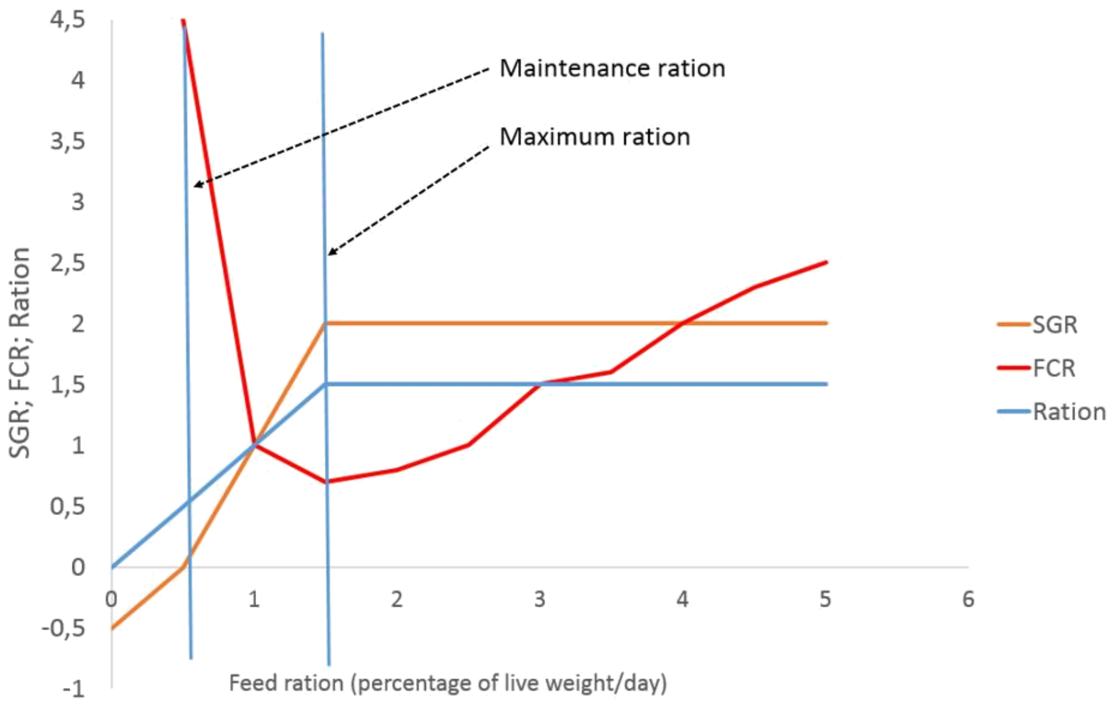


Figure 47: Evolution of specific growth rate (SGR), feed conversion rate (FCR) and ration of feed provided to the fish in terms of the percentage of feed per live-weight of the fish per day

FollowingFigure 47, with a small ration the fish will use all the energy for their daily activities and may even lose weight (where the FCR will be infinite). If we increase the ration, the fish will improve their growth as well as the FCR. At the point of maximum growth, any feed provided in excess will be an economic and environmental problem, with no benefits for production. For that reason, we have to adjust the feed ration to the growth of the fish to a point that is close to the maximal ration, but being careful not to go past that point.

As mentioned above, the control of biological processes involved in aquaculture requires supervision in order to anticipate possible problems. It is important to be able to fix problems as far in advance as possible, which implies detecting very mild symptoms at the outset. All that will help reduce production costs and improve efficiency. As a result, the aquaculture sector understands that it needs to train personnel adequately and continuously, especially those in charge of feeding.

Even in modernised aquaculture systems such as RAS, which are increasingly computerized and automated, personnel need to be aware of the sophisticated biological processes occurring within the unit. Technological developments are increasing but should be accompanied by adequate training in the use of available techniques to improve production on all levels. Those concepts are a foundation for success. Indeed, the continuous training of personnel involved in feeding is a very important tool in farm operations. The supervisor of feeding determines, to a large degree, the profitability of the farm, since he/she provides the energy for the fish to grow. Any changes in feeding habits, however small, can be a symptom of problems in the system which, if uncorrected, can become serious sanitary problems.

1.5.7 Automatic feeders

The automation of feeding requires knowledge about the feeding habits of the species in question. We also need to know technical details, such as the number of fish in each tank and their sizes. Manual feeding has advantages, as mentioned above, and is still used to 'keep in touch' with the fish. Nonetheless, technological developments can facilitate this labour. Nowadays there are many types of automatic feeders, especially for large-scale projects with a large biomass. Here we focus on the different types of automatic feeders used in RAS.

Normally the feed to be distributed is dry and pelleted, and placed directly into the tank where it may float for a time, but eventually it will tend to sink to the bottom. Most fish eat the feed on the surface or on its way down the water column, before it reaches the floor of the tank. Many species used in aquaponics are predators in the natural habitat and show aggressive behaviour when eating, which can lead to problems. Most modern automatic feeders take this fact into consideration, since poor feeding with inadequate feeders can lead to populations with dominant individuals who eat in excess, while the more submissive individuals go without. The immediate consequence is a higher variety of sizes in the tank (more intraspecific diversity in live weight), which makes it necessary to classify it more often, in order to break the social hierarchy and increase feed efficiency. Automatic feeders can be divided into two large groups, related to the biomass of fish and the quantity of feed to be distributed:

1. Feeders for juveniles: These distribute small rations at a high frequency (5-10 times a day). The pellet is very small and feed can be stored directly on the feeder and refilled by hand.
2. Feeders for on-growing: These distribute large quantities of feed at a relatively low frequency (1-3 times a day). Pellets are large and feeders are refilled by hand or automatically.

The cost of manually feeding fish is quite high, both in terms of the tonnes of feed needed as well as the time dedication needed to distribute it. The following company web sites provide details of feeder designs available for different species and aquaculture farms (www.acuitec.es; www.akvagroup.com; www.aquacultur.de). The basic parts of on-growing feeders are:

1. Storage or deposits for different types of pellets which originate from feed bags or silos delivered by truck.
2. Distribution of feed from the deposit onto the distribution site at the tank. Tubing runs from the storage site to the automatic feeder, which in turn has a small deposit. At this stage pellets are moved using mechanical systems or compressors and air injection. This equipment is quite specialized in order to ensure correct supply and adequate hygiene. Examples of the degree of sophistication of the feeding systems used in intensive aquaculture can be found at AKVA group. Some companies also use feeding robots for fingerlings in RAS, which is an automated way to fill up deposits near the tank. The robots move throughout the building using guides or rails that hang from the ceiling (see for example Crystalvision).
3. Distribution site - This is the final part of the automatic feeding system. Here the feed has to be spread out on the surface of the tank all at the same time, thereby allowing all the fish to feed simultaneously, which is better than placing the pellets in one small location.

Thus, the distribution site is important for keeping the tank population more or less homogeneous.

4. Monitoring feed actually consumed - Recent technological developments allow one to detect when fish stop eating, which sends a signal to the automatic feeders to stop providing feed. These systems work with subaquatic cameras or acoustic and laser detectors, which let the feeder know when the appetite of the fish is waning.

1.5.8 Production plan and monitoring the evolution of the farm

All aquaponic farms need well defined production goals and a plan to fulfil those goals. Specifically, it is helpful to define the following aspects well in advance:

1. The species to be used
2. The size of fingerlings needed initially and the target size of the adults to be sold at the end. This will help to define the productive cycles on the farm (types of tanks, etc.)
3. The optimal densities and housing conditions for each stage of growth. This will help to define the maximum load of live biomass in the installation, and annual production
4. The health management to be used to maintain optimal conditions for the fish
5. The level of training of the personnel involved

The welfare of the fish and the economic viability of the installation will depend on compliance with the objectives that are budgeted in the project. We need to know whether fish are reaching their expected growth and transforming feed adequately, and whether mortality is higher than expected. We should know the expected growth curve in relation to the water temperature. That, along with the duration of the production system, will help to design a production plan that will be the basis for the operating costs. Once production has begun, it should be monitored adequately.

There should be clear traceability back to the source of the fish. We need to know the number of fish and their initial size on the first day that they were housed. On a daily basis we register each of the production activities that were carried out, such as the daily source of feed, the cleaning mode, and measures of physical and chemical parameters. In Table 13 we present an example of the control sheet. These data are collected daily for each of the tanks and should be stored in the monthly report and processed in order to be able to determine the evolution of farm production. Periodically we should weigh a sample of fish to estimate growth in each tank. We should capture enough fish to represent the tank, normally at least 10-15 individuals per 100 fish. Feeding is then adjusted periodically according to that average fish weight.

Daily Control Sheet per Tank

Tank number: Treatment:

Number of fish: Average weight: Density:

Source tank: Destination tank:

Day	Temperature (ºC)	Oxygen (mg/l)	Flow inlet (l/s)	Cleaning (partial/complete)	Mortality (number dead)	Feed (g)	Treatments (medical)	Marks	Observations (treatments, movements, incidents)
1									
2									
3									
.									
.									
28									
29									
30									
31									

Figure 48: Data sheet to note details about the tanks and the fish on a daily basis

There are many software control programs on the market, such as those made by the Norwegian company AKVA GROUP, which are used to manage feed. They provide two programs. Fishtalk covers most aspects of control and planning on the farm, as well as production costs. The reports generated and the analysis of the evolution of production are the basis for the decisions to be taken by the manger, both in the short and long terms. AKVAconnect is related to the platform software provided by AKVA GROUP and controls the automation and optimal adjustments of processes and activities on the farm. It offers complete control, with permanent vigilance of the interaction between machines, sensors, and other processes.

Other examples of the information produced and processed during fish production is STEINSVIK for salmon production. In Figure 49 we can see a control screen for the production unit, with physical conditions and growth, fish appetite, fish inventory, the daily rhythm of feeding, etc. For other examples see www.aqua-manager.com.

Finally, as part of the production plan, it is important to maintain feeds under proper storage. Usually feeds are in the form of dry pellets made by extrusion, and hence are relatively easy to store. The quality of the pellets is high and they are quite compact, with limited losses in water since they will not break down easily. To maintain the quality of the dry feeds it is important to store them in silos or in a dry storage area that is insulated from excess heat. If the feed gets humid it can become contaminated with fungi, which in turn produce mycotoxins that can harm fish.

Figure 49:Control screen for the Steinsvik automation program for aquaculture farms

1.5.9 Designing feeds for aquaponics

Fish feeds for aquaponics can be home-made or bought from specialized feed companies that formulate specific diets depending on the species and age of the fish. Normally commercial producers use specialized feeds since they are guaranteed to meet all the nutritional needs of the fish, and tend to be more cost effective compared to making and formulating one's own feed. However, formulated feeds are not always perfect and may have varying effects on the quality of the water where fish live and excrete waste. Only recently have scientists and engineers begun to look at specific diets for fish in recirculation systems and in aquaponic units. Theoretically it seems possible to provide fish with pelleted feed, which will help them to grow quickly, while providing enough nutrients for the plants that will later 'feed' on this water. In practice, however, things are more difficult, and depend on many complex parameters such as the temperature and pH of the recycled water, as well as microbiota in fish intestines and in biofilters. An aquaponics practitioner should know the basics of feed composition in order to have some way to judge which feed would be best to start off with. Although it may not be necessary to design feeds from scratch, students should be able to choose the best feed for this system after reading the following sections.

1.5.9.1 Fish growth and nitrogen retention

The nitrogen that will eventually be eliminated as ammonia by the fish comes from the protein in the feed. Although there is some nitrogen in other components of feed, almost all of the nitrogen absorbed by fish and eliminated as waste is from amino acids since, as their name suggests, they all contain nitrogen in the chemical makeup.

If we know the percentage of nitrogen in the feed, we can then calculate the approximate amount that will be excreted as ammonia into the water by a process similar to that of urination. That ammonia will later be turned into nitrate which will be provided to the plants. It should be noted here, however, that fish do not really urinate but, as opposed to most mammals, they eliminate nitrogenous waste through their brachia (similar to our lungs). In the following sections we will follow the source and fate of nitrogen in an aquaponic system, based on Seawright *et al.* (1998), who were one of the first groups to publish studies on nutrient cycling in aquaponic systems, several decades ago. In their paper they provide an equation for calculating the nitrogen balance in the system, which we will use as a guide. After calculating the nitrogen present in the feed, we calculate how much is retained in the fish, lost as uneaten feed, and lost in faeces, to end up with the concentration of ammonia in the surrounding water.

1.5.9.2 Nitrogen source

Feed is the main nitrogen source in our aquaponic system. In order to calculate the total amount of nitrogen placed into the tank via the feed we first need to know the exact amount of feed used, in grams or kilograms. Next we need to know the percentage of protein in the feed. This is normally shown on the feed label or available from the feed producer. As mentioned in previous sections, fish feeds have high proportions of protein, normally between 25% and 50%. Once we know the protein percentage we can calculate the percentage of nitrogen by dividing it by 6.25. We use that number since nutritionists assume that 1/6.25 or approximately 16% of all protein is nitrogen. Thus, for a feed for tilapia with 35% protein we know it has 35% * 16% = 5.6% nitrogen. If we added, for example, 120 grams of feed to the tank in one day, we are adding 120 * 5.6% = 6.72 g of nitrogen.

1.5.9.3 Nitrogen absorption by the fish

The fish will absorb nitrogen into its protein deposits, which is mostly its muscle. However, most of the fish body weight is water, so that weight has to be discounted since the nitrogen is only present in what can be called the 'dry weight of the muscle'. In general, and based on results in our lab and findings from the literature (e.g., Seawright *et al.* 1998), the dry weight of tilapia is about 27% of its body weight or, put another way, 73% of tilapia muscle is water.

Next we need to know the feed conversion rate (FCR). The FCR is the ratio between the feed provided divided by the weight gained. The inverse of the FCR is called the feed efficiency, or the weight gain divided by the feed ingested. The FCR is typically around 1-2 in fish. The feed efficiency, on the other hand, can be viewed as the FCR divided by 1. That is, for a conversion index of 1.5, the feed efficiency is 1/1.5 = 66.73%. To put it another way, about two thirds of the feed eaten by the fish will be absorbed by the muscle of fish and counted as growth.

Of course it would be better to have a high feed efficiency (close to 100%); the higher it is, the more economically advantageous it is. However, fish have a maximum limit for how much muscle they can accrue over time. As muscle grows, the amount of protein will grow (as well as the amount of total nitrogen in the muscle), but the proportion of protein in the muscle will stay more or less stable. The total percentage of nitrogen with respect to body weight is around 8.8% in tilapia. This may vary among species, but is a good approximate number. So, depending on the feed provided, we can estimate how much nitrogen will be retained in the fish. If we provide 120 g of feed using the values suggested above, then the nitrogen retained in fish will be found by multiplying the feed by the dry weight, by the feed efficiency and by the percentage of nitrogen in fish muscle, i.e., 120g * 27% * 66.73% * 8.8% = 1.90 grams of nitrogen from the feed will stay in the fish.

1.5.9.4 Nitrogen lost in solids

Apart from being lost as urine, nitrogen waste can be lost via faeces. We can measure the protein or nitrogen content of faeces since it accumulates in the solids filter of our system, or we can siphon it up daily and store it. The solid waste could also contain feed that was not ingested but, as mentioned above, it is difficult to measure exactly how much feed was not consumed by the fish, so we lump together faeces and feed not consumed as solid waste. Before analysis, the solid waste is dried in order to calculate the dry weight, and then the nitrogen content is measured. In a RAS system the total amount of solids is around 10%, i.e., 10% of the feed provided to the fish ends up as solid waste (including fish faeces and pellets that are not ingested). When analysed we found that the nitrogen content of the faeces was 4.8%.

As we explained earlier, protein is 16% nitrogen, or that is what nutritionists estimate. Thus, if we only have a measure of nitrogen, to obtain the amount of protein it came from originally we need to 'back-calculate' by dividing the amount of nitrogen by 16%, which is the same as multiplying it by 6.25% (1/16 = 0.0625 or 6.25%). So in the case where the nitrogen content of the faeces was 4.8%, the amount of protein would be 4.8% * 6.25% = 30%.

Finally, to calculate the total grams of nitrogen lost in solids per the amount of feed we provide to the tank, we need to multiply the amount of feed (120 g) by the percentage of feed that is lost in solids (faeces and feed not eaten), and the percentage of nitrogen in the solids (4.8%). Say that the percentage of feed lost in solids is 10%, the nitrogen lost in solids in that case would be: 120g * 10% * 4.8% = 0.576 g of nitrogen in the feed is lost as solids. Again, this is only an example, and that percentage can vary depending on the system and other conditions.

1.5.9.5 Nitrogen dissolved in water as ammonia

Next we can use the above calculations to quantify the nitrogen dissolved in the water, which is essentially lost as ammonia waste. First we add the nitrogen absorbed by the fish and lost in faeces, and then subtract it from the nitrogen applied via the feed. The remaining nitrogen is the amount lost or dissolved in the water. In the case above, 6.72 – (1.90 + 0.576) = 4.24 g NH_3. That is, 63.1% (4.24/6.72) of the nitrogen from the feed is converted into NH_3. It is excreted by the brachia as NH_3 but, depending on the water pH, it is converted into NH_4. The term TAN denotes total ammonia nitrogen, or the combination of NH_3 + NH_4. In Figure 50 we provide an example of results from our lab where the total nitrogen was calculated in feed, and then measured in the fish, faeces, and water.

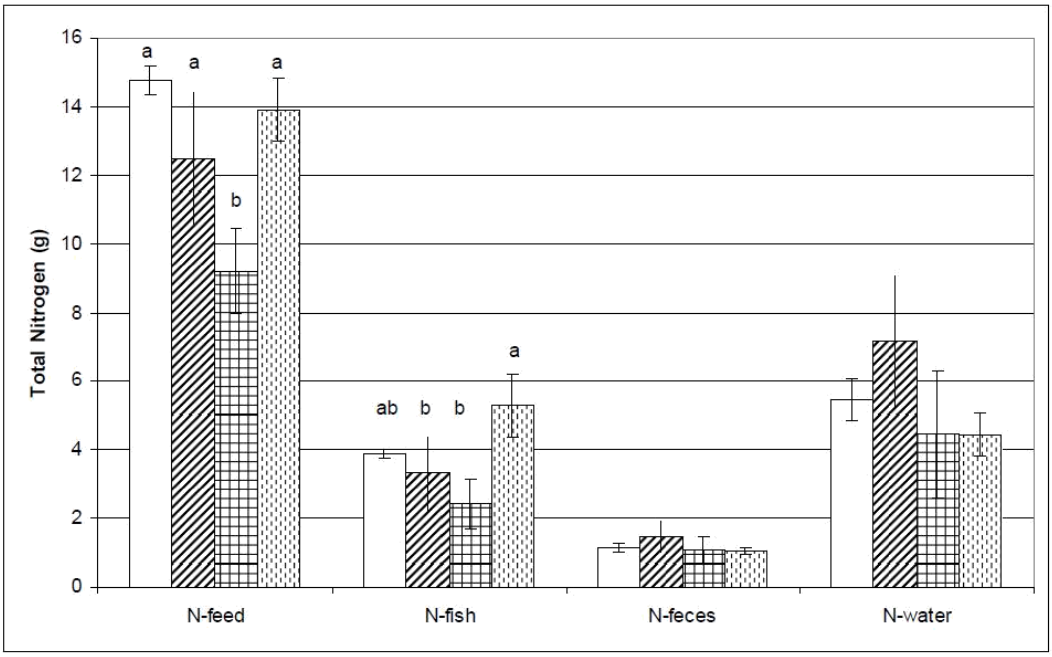


Figure 50: Example of a nitrogen cycle analysis in tilapia using four different feeds based on different protein sources (fish meal, soy, corn gluten, and pea concentrate)

2 Fish biology, health and welfare

2.1 Fish species

There are more than 20,000 species of freshwater and marine fish on our planet, each with specific requirements and ecological niches, which has led to specific body adaptations. However, many of the fish, especially teleost (bony fish with a moveable pre-maxilla), share some common features. Although the number of species used in aquaculture is probably over 200, the number used in aquaponics is narrower, and mostly restricted to freshwater fish (Figure 51).

Common name	Species	Family	Order
Tilapia	*Oreochromis niloticus*	Cichlidae	Cichliformes
Catfish	*Pangasius pangasius*	Pangasiidae	Siluriformes
Koi	*Cyprinus carpio*	Cyprinidae	Cypriniformes
Trout	*Oncorhynchus mykiss*	Salmonidae	Salmoniformes
Bass	*Morone saxatilis*	Moronidae	Perciformes
Perch	*Sander lucioperca*	Percidae	Perciformes
Blue gill	*Lepomis macrochirus*	Centrarchidae	Perciformes

Figure 51: Summary of the species of fish used in aquaponics, including those cited in two international surveys on aquaponic practitioners (Love et al. 2014; Villarroel et al. 2016)

2.1.1 Common carp

Wild common carp, classified as *Cyprinus carpio*, lives in the middle and lower streams of rivers, in inundated areas, and in shallow confined waters, such as lakes, oxbow lakes and water reservoirs. Carp are mainly bottom dwellers but search for food in the middle and upper layers of the water body.

Carp are omnivorous, with a high tendency towards the consumption of animal food, such as water insects, larvae of insects, worms, molluscs, and zooplankton. Zooplankton consumption is dominant in fish ponds where the stocking density is high. Additionally, the carp consumes the stalks, leaves and seeds of aquatic and terrestrial plants, decayed aquatic plants, etc. The pond farming of carp is based on the ability of the species to accept and utilize cereals supplied by the farmers.

Stocking nursed fry is the most effective way for producing medium and large size fingerlings. Common carp can be produced in extensive, natural food and supplementary feed-based monocultural production systems, in stagnant water ponds. Artificial feed-based intensive monocultural production can be carried out in cages, irrigation reservoirs, and running water ponds and tanks, or in recirculation systems. (FAO, 2022)

2.1.2 Rainbow trout

Rainbow trout are classified as *Oncorhynchus mykiss*, and as such belong to the same genus as Pacific salmon, and to the family Salmonidae. Rainbow trout primarily inhabit fresh water, but in the eastern and western North Pacific anadromous stocks are found.

Rainbow trout have been cultured for hundred of years, and are the most widely farmed trout in the world. Can tolerate a wide range of water temperatures and other environmental variables, such as water quality, but they require highly oxygenated water and thrive in water temperatures of 13-18°C. They are a highly valued foodfish, and can be grown to have pigmented (red) or non-pigmentes (white) flesh, depending upon their diet.

In the natural habitat, rainbow trout live in lakes, streams and rivers, consuming zooplankton as fry, followed by insects, crustaceans and other fish as they grow. Spawning occurs in spring associated with the rising water temperature. Rainbow trout growth rate depend on water temperature and food abundance, and wild fish generally reach maturity at 3-4years of age.

Farming systems for rainbow trout are similar throughout the world. Fish are raised in flowing water in earthen or concrete raceways, with stocking densities depending upon water flow and water quality, e.g. temperature and dissolved oxygen content. Water flow is usually gravity-driven. Usually raceways are arranged in series, with flowing from one to another with small drop between the raceways. Upper raceways are typically stocked at higher densities than lower raceways as water quality decreases along a series of raceways.

Rainbow trout feeding is based upon the principal that one should overfeed fry and fingerlings to obtain the fastest growth possible, providing that over-feeding does not pollute rearing water, and feed post-juvenile fish for optimum growth and feed conversion ratios. As with all fish, feeding rates are based upon water temperature and fish size, decreasing as temperature decreases and fish size increases.

2.1.3 Nile tilapia

The Nile tilapia, classified as *Oreochromis niloticus*, has a significant worldwide distribution. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C.

It is an omnivorous grazer that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus. Nile tilapia can filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although its main source of nutrition is obtained by surface grazing on periphyton mats.

Male tilapia grow approximately twice as fast as females. Stocking densities as high as 20 000/m2 have been used if good water quality can be maintained. An initial feeding rate of 20-30 percent body weight per day is gradually decreased to 10-20 percent by the end of a 3 to 4 week sex-reversal period. Rations are adjusted daily, and feed is administered four or more times per day. Brood fish are given high quality feed at 0.5-2 percent of body weight daily.

After sex-reversal, fingerlings are generally nursed to an advanced size before they are stocked into grow-out facilities. This procedure increases survival in the grow-out stage and utilizes growing space more efficiently. Sex-reversed fingerlings are stocked at approximately 20-25 fish/m2 in small ponds and cultured for 2-3 months to an average size of 30-40 g. Final biomass at harvest should not exceed 6000 kg/ha. Fingerlings are given extruded feed (30 percent protein) at an initial rate of 8-15 percent of biomass per day, which is gradually decreased to a final rate of 4-9 percent per day. A recirculation system stocked at 1 000 fish/m3 will produce 50 g fingerlings in 12 weeks. Fingerlings should be fed 3-4 times daily.

2.2 General external anatomy

Most of the fish used in aquaponics follow a basic anatomical outline (Figure 52). Looked at longwise, there are three main regions of the body: the head, the trunk region, and the tail (Canada Department of Fisheries and Oceans 2004). In terms of possible abnormalities, veterinarians tend to focus on problems related to the eyes, fins and skin. Apart from those, there are other parts of the external anatomy that are important in terms of indirect measures of fish welfare, fish quality, and health problems, and one should be able to locate these. For example, blood sampling usually involves injecting a needle underneath the lateral line in the tail region to find the caudal vein. To tag individuals, passive integrated transponder tags (PIT tags) are normally injected into the muscle under the dorsal fin. Some other plastic paints can be injected on or near the mouth and eyes, but any type of exterior tags often cause problems since they affect the very delicate skin and can cause infections. If nothing else, basic knowledge of some species-specific anatomy can also help to avoid fish fraud when purchasing them commercially.

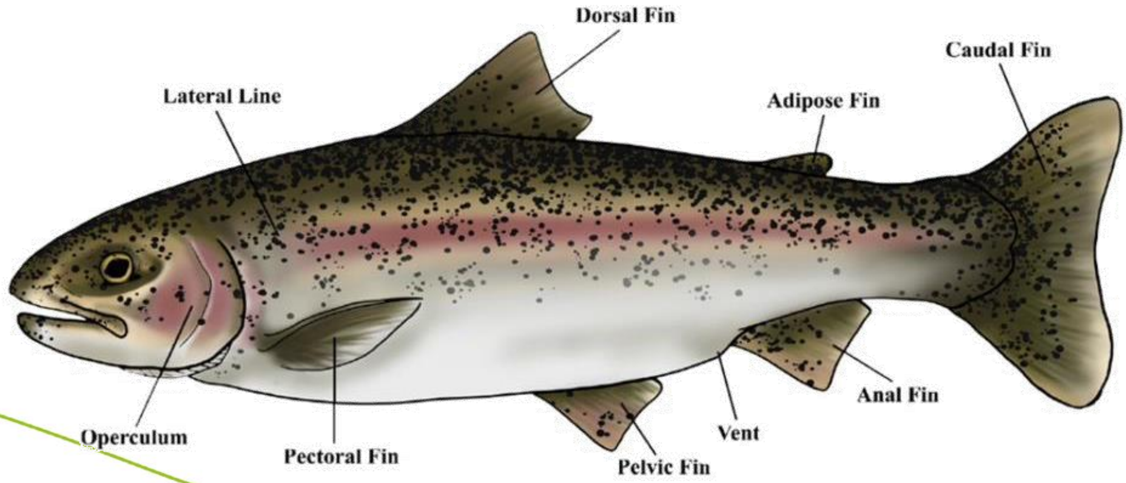


Figure 52: Basic external anatomy of a fish (from http://anatomyhumanbody.us)

2.2.1 Eyes and nose

As opposed to some cartoon characters, real fish have no eyelids. Thus, not only are their eyes in direct contact with the surrounding water at all times, giving an idea of the importance of water quality, they are also quite light sensitive (they have no way of 'closing' their eyes). This is why many fish prefer to avoid direct sunlight and congregate in locations with shade. The Mexican cavefish (Astyanax mexicanus) is one example of a blind fish, but most fish used in aquaponics can see very well. While alive, bilateral exophthalmia (the bulging of both eyes from their sockets) is often used as a general indicator of infection. Unilateral exophthalmia is probably the result of a contusion. After slaughter, the whiteness of the eye is used as a quality indicator (see Council Regulation (EC) 2406/96). For example, a high-grade fish will have a convex eye with a black and shiny pupil, while fish with a concave eye, grey pupil, and a 'milky' cornea should be discarded. Close to the eyes are two small openings (nares) which lead to an area with olfactory sensors which can be quite sensitive in many fish. For example, salmonids use their olfactory sensors during migration in order to return to their original breeding grounds. Technically, in order to be able to smell anything, a current has to be established in and out of the nares, normally while fish are swimming but, unlike in mammals, the holes do not lead to the throat.

2.2.2 Opercula and gills

The operculum is a bony cover that shields the gills, the lungs of the fish which capture the rather limited supply of oxygen dissolved in water. The opercular frequency, or the rate at which the opercula open and close over a period of time, can be used to verify whether fish are breathing correctly or may be overly stressed. In anesthetized or dead fish, veterinarians often 'check under the hood' by lifting up the opercula to examine the gills, which should be bright red and moist, and not covered in mucus, white, or smelly. External observation of the gills can also provide information about possible bacterial or parasitic infections. Compared to mammals, fish lungs are thus more of an external organ than an internal one, again underlining the importance of water quality to protect this delicate and important organ (e.g. correct water pH). Finally, apart from oxygen absorption and CO_2 release, the gills are an important outlet for nitrogenous waste (Figure 53). Hoar & Randall (1984) calculated that more than 80% of ammonia (NH_3) is excreted via the gills, while only trace amounts are passed as urine.

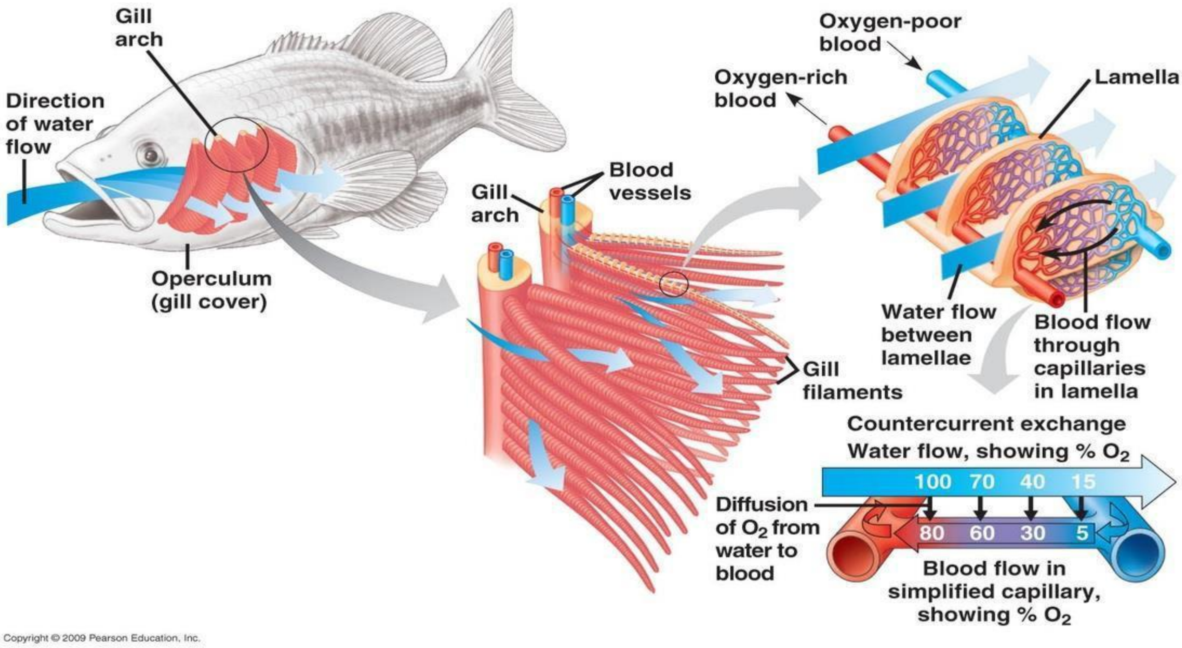


Figure 53: The gills work according to the counter flow principle: water and blood flow in opposite directions.

2.2.3 Skin

The skin is one of the most important organs in fish. It has three basic components: the dermis (inner layer), the epidermis (outer layer), and the scales. The scales are embedded in the dermis, which is responsible for providing colour. Mucus is made by the epidermis and helps to protect the cells. It has anti-fungal and anti-bacterial properties and plays a role in immune function (Wainwright & Lauder 2017). Any type of skin lesion or scale loss can have serious consequences for fish, since healing in an aqueous environment can take a long time and wounds can get waterlogged. Just imagine, for example, trying to heal a paper cut on your finger by keeping it submerged in a glass of water for a week. The whole healing process would take much longer and you would be more exposed to bacterial infections. For all these reasons it is a good idea to use plastic gloves when handling live fish so as not to damage their skin.

The lateral line is part of the skin organ and consists of perforated scales with cilia that are connected to the nervous system and provide information about water movement around the fish and pressure (constituting a sense organ not found in mammals). This allows fish to hunt at night or move in very opaque water by sensing the vibrations around them. The lateral line also has culinary importance, since cutting along this line in a cooked fish will separate the meaty upper section from the visceral section below. Several recent studies have related the colour of the skin on the back of fish (between the dorsal fin and the head) with personality. For example, in salmon, Castanheira et al. (2017) conclude that fish with darker skin or more dark spots in that region are more aggressive.

2.2.4 Fins

Fins can be used as indirect indicators of fish health and welfare. We want to avoid fraying of the fins (when the skin comes apart between the rays), fin erosion (white colouring at the tips of the fins), necrosis (dead cells on the fins), or discoloured spots, the latter of which may indicate the presence of parasites.

2.2.5 Dorsal fin

Normally fish have one dorsal fin, but they can also have two (one after another, as in sea bass). The dorsal fin is mostly used to help maintain the fish in an upright position. It is supported by rays which are often erectile to allow the fish to 'open or close' it depending on signaling requirements. Tilapia has a large dorsal fin with pointed rays that can easily cut innocent hands that want to grab it out of the water. The number of rays per fin can also be used to identify the species of fish. For example, rainbow trout have between 10-12 rays on their dorsal fin while brown trout (not normally grown in aquaponics) have around 13-14.

2.2.6 Adipose fin

This is a rather short and fat fin which is common in salmonids, but whose function is unclear. It is full of fat and appears to have sensory neurons. Sometimes it is cut off in farmed salmon to differentiate them from wild salmon but Reimchen & Temple (2004) found that fish without an adipose fin have a higher tail beat amplitude, indicating that it has a role in natural swimming behavior, and that cutting it off probably has a negative effect on welfare.

2.2.7 Caudal fin

This is the largest and most powerful fin and is directly connected to the spine. It is used to thrust the fish forward. Like the tail of piglets, it can also be nibbled by other fish or get eroded by being rubbed on different surfaces. The tail is also important for measurement purposes (Figure 53). Apart from weighing the fish, aquaculturists often measure the standard length (from mouth to the beginning of the tail) and fork length (from mouth to the fork at the tip of the tail).

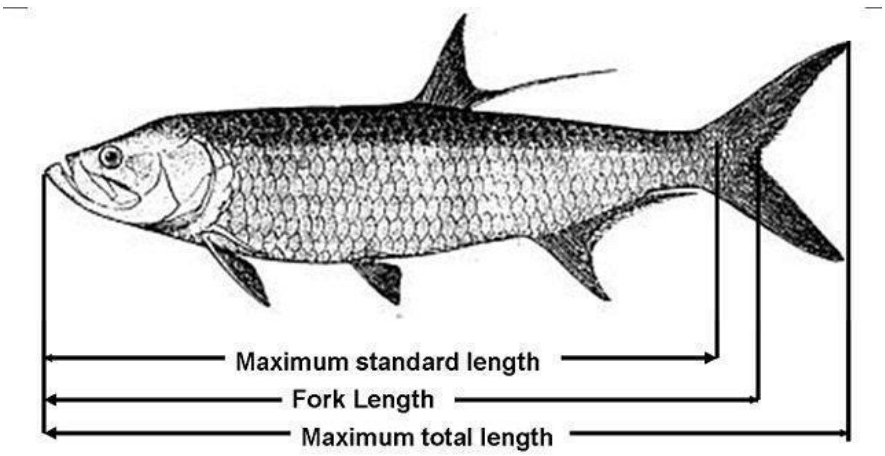


Figure 54: Example of fish length measurements for a tarpon fish. For standard weight equations, the total length is used (source http://www.nefsc.noaa.gov/lineart/tarpon.jpg)

2.2.8 Anal fin

This fin is posterior to the anus and urogenital pore.

2.2.9 Pectoral and ventral fins

Close to the operculum fish have pectoral fins, which roughly correspond with the arms of terrestrial mammals, and below them are the ventral or pelvic fins, which roughly correspond with 'legs'. In some fish, generally those considered to be 'less evolved' (i.e. those which have changed less over time compared to their ancestors), like salmonids, the ventral fins are further down the trunk region, while they are closer together in more modern fish (such as tilapia). The pectoral fins help fish to move up and down while the ventral fins are more important in stopping movement.

2.3 General internal anatomy

In this section we will outline the most important internal organs of fish (Figure 55), underlining the main differences with mammals and some important facts that influence how fish should be maintained.

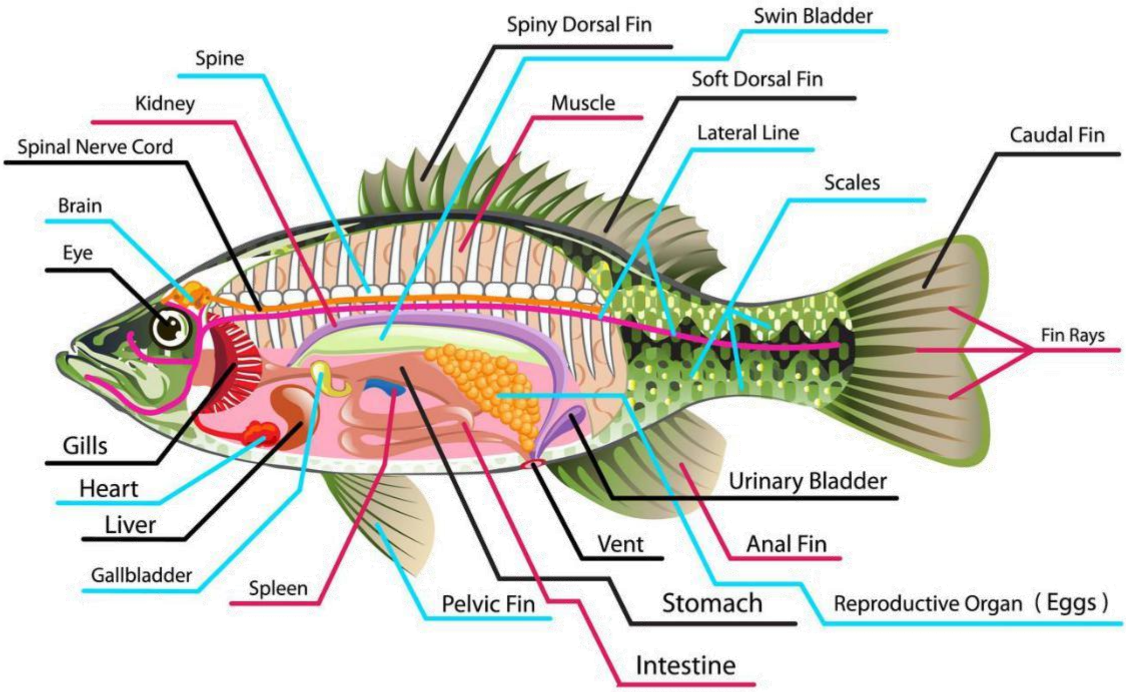


Figure 55: General internal fish anatomy (source http://www.animalsworlds.com/internal-anatomy.html)

2.3.1 Brain

Fish have small brains compared to terrestrial vertebrates. For example, the human brain weighs approximately 1.4 kg and represents around 2% of the total body mass, but fish brains only represent 0.15% of their body mass. Nonetheless, unlike many vertebrates, fish brains are quite adaptive and maintain the ability to grow and change throughout life (they maintain the ability to produce new neurons; Zupanc 2009). Fish brains have three main regions: the forebrain (with the olfactory lobes and telencephalon), the mid-brain (optical lobes), and the hind-brain (cerebellum). Fish do not have a neocortex, which some scientists think is necessary to be fully conscious of pain, but other important structures exist that suggest they can feel pain, such as the amygdala, the cerebellum, and the pallium (outer layer of the telencephalon; for more information see Braithwaite 2010).

2.3.2 Heart

The heart is located just underneath the gills. Like the brain, it is quite small and relatively simple compared to terrestrial vertebrates, normally only weighing a few grams. It has a contractile ability to collect blood from the body and send it to the gills in a one-loop system which will be commented on more below under the section on respiration. It is a simple circuit with one atrium, one ventricle, and a conus which leads directly to the gills. There is no double circuit as in mammals, where the blood sent to the lungs returns to the heart to get pumped back to the body. In fish the gills 'pump' the blood to the body without sending it back to the heart.

2.3.3 Digestive system

The general makeup of the digestive system in fish is similar to other vertebrates, with a mouth, esophagus, stomach, small intestine, large intestine, and anus. However, there is little demarcation between the different sections of the small intestine, nor is there an ilea-caecal valve separating the small from the large intestine. Carnivorous fish (like salmon) have a simple and short stomach while herbivores (such as carp) may lack a stomach altogether and have a longer intestine with more pyloric caeca. The caeca are derivations of the digestive tract, which help to increase the total surface area for digestion and extract essential nutrients.

2.3.4 Intraperitoneal fatty tissue

An important difference between wild and cultured fish is the amount of fat that accumulates in the intraperitoneal cavity in the latter. The peritoneum is the membrane that lines the abdominal cavity and the intraperitoneal cavity is the space between the peritoneum that surrounds the abdomen and the peritoneum that surrounds the internal digestive organs. For example, sea bream from aquaculture will typically accumulate more intraperitoneal fat than wild sea bream, while fish that are fasted for longer periods have less fat than fish fasted for less time (Mozanzadeh et al. 2017). Intraperitoneal fatty tissue is a white colour that clings to the intestine. This is a problem since it contains rich sources of omega 3 fatty acids found in the feed and is essentially wasted since consumers normally do not eat it.

2.3.5 Spleen

The spleen is normally a dark red circular organ attached to the intestine. It helps to clean the blood, contains white blood cells, and is an important part of the immune system.

2.3.6 Liver

The liver is quite large and reddish, and beginners sometimes confuse it with the heart. It plays a vital role in detoxifying any organic or inorganic contaminants found in the food or water, as well as participating in protein synthesis, and fat and glycogen storage. Underneath the liver is the yellowish green gallbladder. Most fish do not have a distinguishable pancreas but rather Brockmann bodies, a collection of endocrine cells found along the digestive tract which can produce insulin.

2.3.7 Swim bladder

This organ is unique to fish. It can be filled or emptied to control buoyancy, and thus affects the amount of energy needed to swim. It can also be used to produce or receive sounds. Fish can be either physostomous (like trout), who can fill up their swim bladder via a pneumatic duct which is connected to the gut, or physoclistous (like bass), with no direct connection between the oesophagus and the entry to the swim bladder, so it must be filled up using a gas gland. Physostomous fish are better prepared for sudden changes in water height while it will take longer for physoclistous species. For all fish it is important to fill the swim bladder with air at an early stage of development, in order to assure proper growth and avoid spinal deformities (Davidson et al. 2011).

2.3.8 Kidneys

The kidneys are paired organs that are quite long and narrow, and dorsal to the swim bladder. They play an important role in blood homeostasis (i.e., maintaining appropriate levels of dissolved ions), which explains their substantial size. As in mammals, they are needed to 'clean' the blood, which is especially important in an aqueous medium where the concentration of different ions must be monitored continuously. It should be noted here that fish from fresh- and saltwater have adopted opposing methods to maintain appropriate levels of blood electrolytes. Freshwater fish have a higher concentration or ions in their blood and the surrounding water. Therefore, due to osmosis, the gills and kidneys of those fish must work to avoid absorbing too much water (H_2O) and losing too many ions (they drink little and 'urinate' a lot). In saltwater the opposite occurs: fish drink/ingest more water and urinate little since the concentration of ions in their blood is lower than the surrounding water. In aquaponic units, care should be taken to ensure that the nutrient solution for plants is not having a negative effect on the fish due to inappropriate ion levels. At the end of the kidney there is a bladder to store urine, but it is very small compared to mammals, mostly because little urine is produced in comparison (as mentioned above, much of the nitrogenous waste is excreted by the gills).

2.3.9 Testes and ovaries

Most of the fish used in aquaponics will be used as food and will not mature sexually (breeders are kept in a separate installation). However, it is useful to know that sexual reproductive organs in fish are internal and start to develop deep inside the dorsal region of the fish near the head kidney. As fish mature, the gonads grow in size drastically towards the urogenital pore near the anus. During breeding season semen or eggs will be expelled for external fertilization.

2.4 Respiration physiology

The air we breathe is mostly nitrogen (78%) and 21% oxygen. The water that fish 'breathe' also contains oxygen, but at a much lower concentration, less than 1%. In addition, since water is 840 times denser than air and 60 times more viscous, it takes more effort for fish to 'breathe' to extract oxygen, around 10% of their metabolic energy. In comparison, terrestrial animals only use about 2% of their metabolic energy to extract oxygen from air. For example, rainbow trout need to move approximately 600 ml of water past their gills per minute per kg weight while, in comparison, terrestrial reptiles such as turtles only need to move 50 ml air min-1 kg-1. As a result, even though fish gills are quite efficient, obtaining enough oxygen from the surrounding water can be difficult and sometimes life threatening.

Fish capture oxygen using their gills which are in direct contact with the surrounding water and are easy prey for parasites and bacterial infections. The total surface area of the gills is approximately 10 times the surface area of the whole body. Gills are also important in ion exchange (maintaining the acid-base balance) and waste elimination, such as ammonia. Thus, fish basically urinate via their gills as well as breathe through them. To obtain oxygen, water is drawn into the mouth cavity and then the mouth is closed to force water out through the two opercula. This pumping movement creates a unidirectional flow of water, unlike the inhaling and exhaling through the same orifice in terrestrial mammals. Some fish, such as sharks, can keep their mouth open while swimming, which apparently provides enough flow of water over the gills to breathe normally. If your tanks allow it, you can try to measure the heart frequency of your fish indirectly by counting the opercular frequency – the times that the opercula open and close during one minute. This measurement can be used as an indirect indicator of animal welfare since stressed fish have high opercular frequencies.

Most fish have four gill arches on each side of their body. Each arch consists of a white bony rod which runs from top to bottom (ventral-dorsal) from which stem the V-shaped primary filaments in a caudal direction. The primary filaments or primary lamellae are red since they are full of blood. Each primary lamella has secondary lamellae which cross it perpendicularly and carry individual blood cells to facilitate gas exchange (release CO_2 and capture O_2 using the haemoglobin in the red blood cells). The flow of the blood runs against the flow of water, which increases its efficiency. In addition, fish can open or close the set of primary filaments to expose more secondary lamellae to the water, effectively taking deeper breaths. After filling up with oxygen the blood cells continue to move through the body via arteries.

2.5 Culture environment – physical parameters

2.5.1 Temperature

Temperature is an important factor effecting the growth and survival of all organisms. Water temperature is especially important to the growth and survival of fish, crustaceans and other aquaculture animals, because they are poikilothermic or coldblooded. Poikilothermic animals cannot control their body temperature, and they equilibrate with the temperature of the surrounding water. Aquaculture animals are usually classified as cold water, warmwater, and tropical species:

- Coldwater species will not tolerate temperatures above 20 to 25 °C
- Warmwater species will usually not reproduce at temperatures below 20 °C or grow at temperatures bellow 10 to 15 °C, but they survive much lower winter temperatures.
- Tropical species will die at temperatures of 10 to 20 °C, and most do not grow at temperatures below 25 °C.

Temperature ranges given above are very general, and each species, whether cold-water, warmwater, or tropical, has its characteristic temperature requirements. The temperature effects on a tropical species of fish is illustrated in Figure 56. There is a low temperature below which fish die. At slightly higher temperature the fish live, but they do not grow or grow very slowly. At a certain temperature, growth will increase rapidly with increasing temperature until the optimum temperature is reached. As temperature rises beyond the optimum temperature, growth will slow, cease, and fish will die if the increase continues.

The relationship in Figure 56 is slightly different for warmwater or cold-water species. These organisms are not likely to die as a result of low temperature in natural or aquaculture waters. (Boyd, 2018)

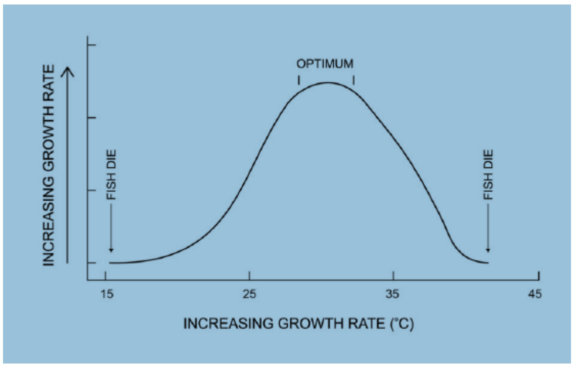


Figure 56: Water temperature in aquaculture. (Boyd 2018)

2.6 Fish health

Monitoring fish health is a central aspect of keeping a healthy aquaponic system. It is typically achieved through observation of the behaviour and physical appearance of stocks, and an understanding of what constitutes 'normal'. The term water quality includes anything that adversely affects the conditions required for maintaining healthy fish. Maintaining good water quality in an aquaponic system is of extreme importance. Water is the medium through which all essential macro and micronutrients are transported to the plants, and the medium through which the fish receive oxygen; therefore, it will directly affect the productivity and viability of the system. There are five key water quality parameters that are crucial for close monitoring in the system: dissolved oxygen (DO), water acidity (pH), water temperature, nitrogen compounds (ammonia, nitrites, and nitrates) and water hardness. Other parameters also need to be monitored in order to maintain a healthy balanced system, such as phosphorus and other nutrients, algae contamination, suspended solids, carbon dioxide concentration, etc. However, these parameters can be monitored less frequently in a well-balanced system.

- Effects:
  - A stress-free condition will help to ensure that fish maintain a healthy immune system, which will allow them to fend off complications arising from the introduction of disease and parasites.
- Monitoring:
  - Daily observation of the fish
  - Note the body condition and any change
  - Observation of clinical signs of stress, disease and parasitic infestation.
  - Water quality maintenance.

2.6.1 Chemical parameters

3.6.1.1 pH - The pH of a solution is a measure of how acidic or alkaline it is on a scale from 1 to 14. pH < 7.0 is acidic, pH = 7.0 is neutral and pH > 7.0 is alkaline. pH is defined as the amount or the activity of hydrogen ions (H^+) in a solution. pH is lowered as the hydrogen ion activity rises, this means that acidic water has high levels of H^+ and then low pH. Fish have a pH tolerance range from about 6.0 to 8.5. In order to satisfy the needs of all organisms, the pH in the aquaponic system should be kept somewhere between 6.0 and 7.0.

The processes of nitrification, fish stocking density and phytoplankton contamination will affect the pH of the system, so it will not stay constant and will need to be regularly monitored.

- Nitrification process - bacteria produce small concentrations of nitric acid and the pH of the aquaponic system is lowered.
- Fish stocking density - when fish respire they produce CO_2 which is released into the water, upon the contact, CO_2 is converted into carbonic acid (H_2CO_3), which also lowers the pH of water. This effect is greater at higher fish stocking densities.
- Phytoplankton – for the photosynthesizes uses the CO_2 in the water and raises the pH, especially during the day when photosynthesis is at a maximum (Somerville et al.2014a; Thorarinsdottir et al.2015).
  - Effects of different values of pH
    - Nitrifying bacteria are unable to convert ammonia into nitrate at pH of 6.0 or below - biofiltration less successful and ammonia levels may begin to increase.

  - Monitoring
    - pH test strips - where the test strips are submerged in the water and compared to the colour code on the bottle or box - moderately accurate.
    - Water testing kits - which come with reagents and a colour-coded chart for comparison - next level of accuracy.
    - Digital meters with pH probes and on-line monitors - for continuous monitoring - most accurate method.
  - Problem solving procedure:
    - Raise the pH
      - Adding gradually $NaHCO_3$ dissolved in water whenever needed. Normally up to 20g per 100L. Do not add too much at one time as this can kill the fish.

  - Strong bases such as calcium hydroxide ($Ca(OH)_2$), or potassium hydroxide (KOH) - dissolve the pellets or powder in water and add it gradually to the fish tank.
- Lower the pH
  - Add gradually acid in the reservoir (never directly to the fish tank!) such as phosphoric acid (H_3PO_4), which is a relatively mild acid (Thorarinsdottir et al.2015).
- $NaHCO_3$ can also be added in the tank every day, as a preventive measure, always taking into account the value of the pH daily.

2.1.1.2 Dissolved oxygen

Dissolved oxygen describes the amount of molecular oxygen in water and is usually measured in milligrams per liter (mg/L).

- Effects of different values of DO
  - Levels are not sufficient - fish are under stress or suffer from slow growth and could die.
  - High DO levels - required by the nitrifying bacteria in the biofilter, to convert fish waste into plant nutrients.
  - DO requirements differ for warm water and cold water fish.
  - It is recommended that DO levels be maintained at 5mg/L or higher in an aquaponic system.
- Monitoring
  - Should be measured frequently in a new system, but once procedures become standardized it is not necessary to measure DO quite as often.
  - Aquarium kits that include reagents for testing DO content, but the most reliable approach is using DO probes with electronic meters, or online monitors that constantly measure the most significant parameters in the fish tank.
  - In a small-scale unit it might be sufficient to frequently monitor fish behavior, water and air pumps instead - fish coming to the surface for oxygen-rich surface water, this indicates that DO levels in the system are too low.
  - Ensure that the water and air pumps are constantly circulating and aerating the water.
  - Low DO levels are not usually a problem with hobby aquaponics growers using low fish stocking rates. The problem tends to arise more in operations with high stocking rates.
- Problem solving procedure:
  - If DO levels are too low - increase aeration by adding more air stones, or by switching to a larger pump (there is no risk of adding too much oxygen).
  - DO levels are closely related to the temperature of the water - cold water can hold more oxygen than warm water, so in the event of warmer weather, the monitoring of DO or preventively increasing aeration is essential.

- Oxygen consumption is also related to the size of the fish - smaller fish consume considerably larger amounts of oxygen than large fish. This fact needs to be taken into consideration when setting up the system and stocking with small fish (Sallenave 2016; Somerville et al.2014a).

2.6.1.1 Total nitrogen: Ammonia, Nitrate and Nitrite

Nitrogen compounds are crucial water quality parameters. Ammonia and nitrite levels should be close to zero, or at most 0.25–1.0 mg/L.

- *Effects of* different values of nitrogen compounds:
  - High level can be toxic and even deadly if the fish are exposed for long time
- Monitoring:
  - Performed daily or at least weekly in order to keep an eye on ammonium and nitrite peaks.
  - Aquarium kits for ammonia, nitrite, and nitrate - quite accurate and cost efficient.
  - Spectrophotometers, combined with laboratory chemical analysis - more accurate measurements
- Problem solving procedure:
  - If nitrite or ammonia peaks occur, do not feed the fish for several days.
  - If the levels are critical, immediately flush the system with fresh water

2.6.1.2 Phosphorus content

Phosphorus is one of the few minerals which fish cannot supply their metabolic needs from via the water. In the past, phosphorus deficiency was uncommon because fish feeds were largely fishmeal-based, which are high in phosphorus levels. There has long been concern, however, not only for the long-term availability of fish meals but also for phosphorus pollution from fish farm effluents. For these reasons, the trend has been for more sophisticated diets with proteins of plant origin and high-energy levels. Fish that are deficient in phosphorus do exhibit the typical signs of anorexia and dark coloration, but there are also very specific clinical signs of phosphorus deficiency associated with its primary role in bone mineralization, such as deformities of the head, ribs, and vertebrae.

2.6.1.3 Water hardness

There are two types of water hardness which are especially relevant for aquaponics: general hardness (GH) and carbonate hardness (KH). GH is described as the amount of calcium (Ca^{+}), magnesium (Mg^{+}) and, to a lesser extent, iron (Fe^{+}) ions present in water. KH can be described as the total amount of carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{-}) within a system, which gives water alkalinity. The optimum hardness level for aquaponic systems is between 60-120mg/L (moderately hard).

Water hardness can be classified as: Soft 0-60mg/L; Moderately Hard 60-120 mg/L; Hard 120-180 mg/L; Very Hard >180 mg/L.

- Effects of different values hardness:
  - GH is important for both plants and fish within aquaponic systems, as Ca^+ and Mg^+ are essential plant nutrients and are therefore required for healthy plant production. It can also be a useful source of micronutrients for fish within the system; for example, Ca^+ within the water can prevent fish from losing other salts, thereby increasing the overall productivity of the system.
  - KH therefore has an impact on pH levels, and acts as a buffer to increased acidity which can arise from certain physiological processes such as nitrification.
- Monitoring:
  - In RAS systems should be monitored once a week.
  - Test strips - should be placed in the water and it will change colour depending on the degree of hardness and then comparing to the colour charts provided in order to get the corresponding level of hardness.
- Problem solving procedure:
  - If it is found that the water is not at a suitable level of hardness, it is often possible to fix this with additives to increase the level. Limestone or crushed coral can also be added to water to increase hardness (Sallenave 2016; Somerville et al.2014a;Thorarinsdottir et al.2015).

2.6.2 Biological parameters

2.6.2.1 Density of the fish

Stocking density is a very important factor that has to be decided in advance when designing a RAS. Different fish species have different possible stocking densities. Density is a central factor in determining fish welfare, although all the biological aspects are not clear yet. There are fish species that have different behaviour at different densities. For example, tilapia adopts schooling behaviour at high densities, and territorial behaviour at low densities. In order to prevent fish harming each other, they therefore have to be farmed at a certain density. To use space efficiently, and to prevent cannibalism, a fish tank should contain fish of approximately the same size. This means (a) that an aquaculture facility should have several tanks to house fish of different size classes, and (b) that the fish population has to be graded according to size occasionally, and redistributed into the tanks.

nfluencing factors for systems with the same annual production	High density	Low density
Change water parameter Response time (for example to pump failure)	Fast change Is shorter. More stress for the fishes	Slow change Is longer. The system operation is safer
Capacity of the fish tanks for a given production volume	Less capacity for the same production volume	Higher capacity needed. This can be compensated partly by using deeper basins. However, these are more expensive and need a more expensive pipe and pumping system
Necessary circulation/displacement rate for a given production (m^3/h)	Same	Same. Due to the slowness of the system, there are softer peaks = smaller components = less expensive hardware for water reconditioning
Displacement volume relative to tank volume	High	Low
Tank dimensions	Smaller tanks with a high density of individuals are, depending on the species, more prone to stress	In larger tanks, easily scared fishes have a longer escape distance

Figure 57: Characteristics of low and high stocking density systems

2.6.2.2 Feeding rate

Important to improve feed efficiency and can increase aquaculture sustainability through reduced feed costs and environmental impact. Growth is an important measure of how well fish are doing in a system, and feed companies often provide growth charts which give an estimation of the expected growth rate of fish as a function of feeding rates. Moreover, a restricted feeding rate reduces the work load of collecting uneaten pellets after each meal.

- Effects: individual phenotypic information is required.
  - Too much food can lead to:
    - Oversupply of nutrients in the water - complications in the chemical and micro (biological) parameters.
    - Increased bacterial load - can allow causative agents of disease to take a foothold.
    - Increase in the biochemical oxygen demand, and changes in other chemical parameters, such as pH.

- Too little food can lead to:
  - Stunted growth - decreased productivity in the system,
  - Increased stress and aggression - can cause fish to attack each other, resulting in wounds and sores which may become infected.

- Monitoring:
  - Weighing the quantity of feed
  - Feed ingrates can also be measured visually, by monitoring the fish until feeding rates decrease and they cease feeding;
  - Some systems use underwater cameras to monitor the fish
  - Many fish feeding companies will give recommended feed rates, allowing operators to accurately estimate how much feed to give.
  - Feeding rates should be observed and noted at each feeding to allow for monitoring.
- Problem solving procedure:
  - If feeding rates begin to reduce, this could be a sign that something is wrong in the system and appropriate action, such as investigation by a veterinarian, should be undertaken. (Masser et al.2000).
  - An increase in feeding rates could be a sign that the fish are not being fed enough, in which case the feed should be increased (Masser et al.2000).
  - Growth is measured physically, by first weighing and then measuring the fish. These measurements should be taken once a week and noted. Any unexpected change to size and weight should be investigated.

2.6.2.3 Algae contamination

Algal growth in an aquaponic system can have negative effects on its performance. Algae are photosynthetic organisms, and will quickly and easily grow in water if exposed to light. Since they occur naturally in all sources of water, it is almost inevitable that they will occur within an aquaponic system. Algal physiology varies between single celled organisms, known as phytoplankton, and multicellular types, known as macroalgae. Each of these comes with their own problems, as phytoplankton can reproduce rapidly, turning water green, while macroalgae form long filamentous strands, which can attach to the bottom of tanks

- Effects:
  - Can affect the chemical characteristics of the water.
  - Can interfere with the mechanics of the filters and pumps.
  - Will compete with your target species for nutrients. As they are photosynthetic, they will also act as a sink for DO, producing oxygen during the day, and consuming it at night. In serious cases, algal consumption of oxygen during the night can result in water becoming anoxic, causing fish death.
  - Filamentous algae can also grow to quite large sizes, and are often tough to break down. This means that a buildup of algae can cause damage to the filters and pumps

which may be expensive to repair and which can compromise the performance of the system.

- Monitoring:
  - Visual inspection of the system in areas such as the walls of fish tanks, around pumps and filters, and around the roots of the plants.
- Problem solving procedure:
  - Blocking light using screens will prevent their growth.

2.7 Fish welfare

2.7.1 Introduction

Aquaculture is one of the few types of animal farming that has grown continuously over recent decades, by about 10% annually on an international level (Moffitt & Cajas-Cano 2014). However, as production increases and new methods appear, such as aquaponics, we have been witness to more problems related with fish health and welfare. Although it may seem surprising, more than 1300 scientific articles have been published on fish welfare since 1990 (Table 4). Not all those studies deal with commercially produced species, but in general the number for all fish is comparable to or higher than some other species like sheep, horses or poultry.

Species	Papers
Fish	1295
Trout	550
Sheep	1149
Cattle	2417
Pig	2638
Horse	926
Poultry	1078

Figure 58: Summary of publications on animal welfare for different species of farm animals (based on a search in the Web of Science for the years 1990-2017

One of the first scientific reviews of fish welfare was by Conte (2004) from the University of California at Davis, followed a few years later by two groups from the United Kingdom (Huntingford et al. 2006 and Ashley 2007). In his review, Conte (2004) underlines that fish farmers already know that welfare is important and that stress must be minimized since fish have specific requirements in terms of handling and environment outside of which they will not thrive or survive. That is to say, compared to terrestrial animals, fish are more demanding in terms of growing conditions and can be stressed easily, so much so that they can also die easily. Huntingford et al. (2006) summarize the main arguments for believing that fish can feel pain. Fish are complex beings that develop sophisticated behaviour, so the authors believe they can probably suffer, although it may be different in degree and type than for humans. That review ends up identifying four main critical areas when considering fish welfare: assuring that fish are not kept without water or food; assuring that producers provide good water quality and equipment; that their movements or behaviour are not restricted; and that mental and physical suffering be avoided. In his review, Ashley (2007) starts with a description of the industry and the critical points that may compromise fish welfare, including fish density in cages and problems with aggression. For example, some species, like tilapia, are more aggressive when kept at low densities than at higher densities. Importantly, Ashley (2007) provides a table of the main welfare problems in fish which is 7 pages long. In conclusion, there is a lot of scientific literature about fish welfare and several critical areas have been identified. However, regarding aquaponics, there are very few studies about the welfare of fish bred together with plants, but we can learn from other studies about the welfare of fish kept in small-scale recirculation systems.

2.7.2 Legislation in the EU

In Europe, any animal kept for the purpose of farming must comply with Directive 98/58/EC, which is a law that sets down several minimum conditions for adequate animal welfare for vertebrates. Although fish are technically included in that Directive, they are practically exempt due to our lack of knowledge about fish welfare, so there are no specific requirements for minimum conditions for fish used in aquaculture. Since 2006, several reports have been published in Europe, for example by the European Council of the European Food Safety Authority (EFSA), which give scientific recommendations for the most common species used in aquaculture. Overall, at least in Europe, there seems to be general agreement that fish undergo stress when oxygen levels are low and when they are taken out of water, and that chronic stress in fish compromises the immune system and can make them more vulnerable to disease.

2.7.3 Specific measures to evaluate welfare

Studies on fish welfare began later than for other farm animal species, in part because aquaculture is a younger animal production science and also since it was unclear to many whether fish can feel pain. Until recently, fish were not considered to be sensitive animals, but that situation has been changing. Sneddon (2003) was one of the first to prove that trout have pain receptors (nociceptors) on their face and jaw. They proved that those receptors respond to stimuli which are potentially damaging and send nervous signals to the spinal cord and brain. In addition, it appears that trout are aware of pain since they change complex behaviour when given a noxious substance, but revert to normal behaviour when given morphine (which essentially eliminates the pain). Those findings have also been confirmed in other species such as goldfish, where anxiety and fear decrease when they are given doses of morphine (Nordgreen et al. 2009). On the other hand, other scientists like Rose (2002) argue that fish cannot feel pain like humans since they lack a neocortex. Thus, they are probably not conscious about their pain in the same way as we are, although they react to pain in a similar manner. Whatever the case may be, both sides agree that fish can be stressed and that they have evolved a complex physiological response to stressors. Dawkins also makes the important point that everyone should worry about animal welfare whether or not they are conscious, simply because poor animal welfare leads to diseased and unhealthy fish, which has negative effects on farmers and consumers (Dawkins 2017).

2.7.4 The HPI axis and the stress response

The cascade of neuroendocrine activities that are released in fish after they become aware of a stressor are very similar to the responses seen in other vertebrates. As in mammals, the immediate neuroendocrine response is called the primary response and consists of nerve signals which release adrenaline and noradrenaline from chromaffin cells (at the head kidney), whose equivalent in mammals is the adrenal medulla (Figure 59). After the primary response there is a slower secondary response which takes 2-15 minutes to activate the hypothalamo-pituitary-interrenal axis, or HPI (Sumpter et al. 1991). The hypothalamus produces corticotropin releasing hormone (CRH) which stimulates the production of cortisol by the interrenal tissue (also associated with the kidneys), which corresponds with the adrenal cortex in mammals (Okawara et al. 1992). The secondary response includes an increase in heart frequency, greater oxygen uptake by the gills, and an increase in glucose concentration in plasma via glucogenolysis (Pickering & Pottinger 1995).

Although there is no simple relationship between stress and welfare, we know that they are related and that the response to a stressor can be used to give an idea about the degree of the challenge. With that in mind, it is always preferable to consider several indicators at the same time, including growth indices, immune system response, and other physiological indicators.

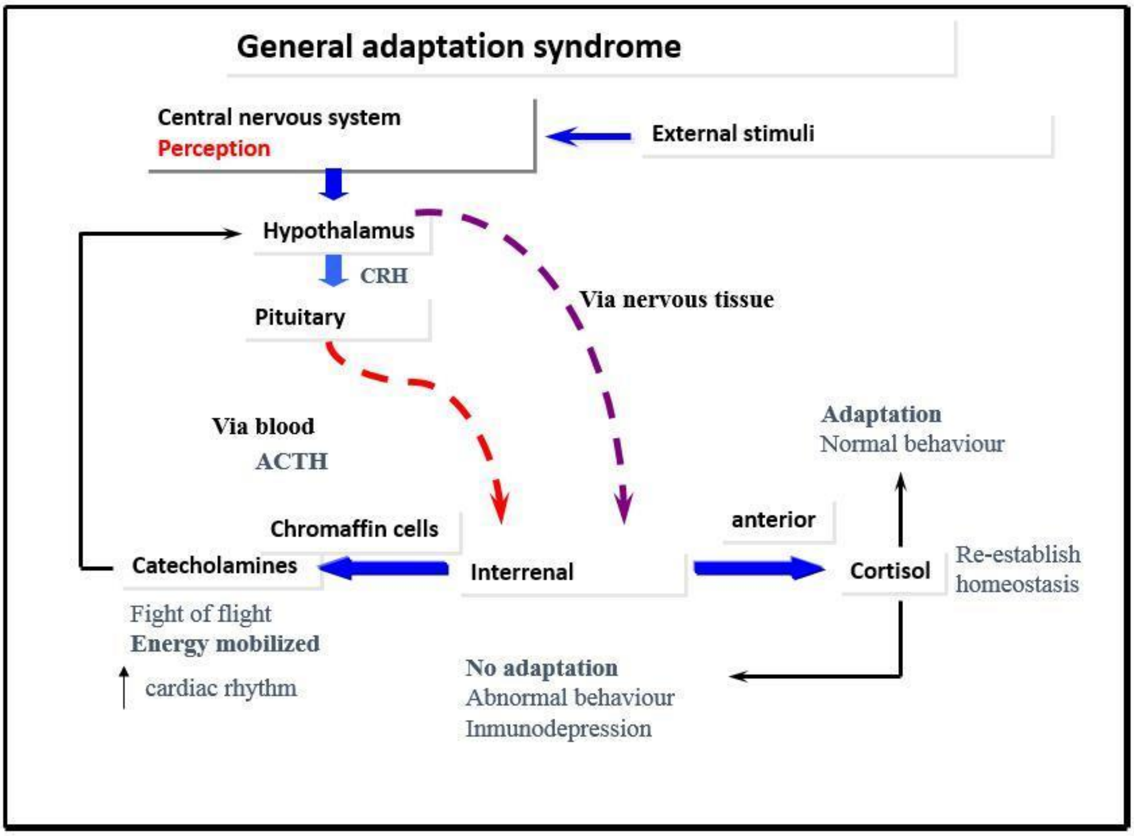


Figure 59: The HPI axis in fish and the cascade of responses to a stressor (source M. Villarroel)

2.7.5 Operational welfare indicators

On an industrial level, a new approach is being developed to analyse fish that involves interactions between scientists studying animal welfare and companies that strive to be more efficient. Together they are developing operational welfare indicators (OWI). A good example for salmon is the manual presented by Noble et al. (2018) that tells farmers how to evaluate on a commercial level the immediate environment, different groups of fish, and individual fish. As mentioned above, many scientific articles have been published on fish welfare, most of which are based on observations made in the lab. OWI are practical indicators that are used on the farm and can be easily explained and repeated. OWI can be separated into two large groups: those more related to the environment; and those related to the fish. The latter can be applied to groups of fish, or individually. Finally, individual indicators can include laboratory analyses which are less operational per se but can provide useful information in the short term (Figure 60). OWI can provide an idea of the current status of production in terms of the needs of the fish and their welfare. In parallel, they can be used to help develop good practice and to identify critical points that can have a negative effect.

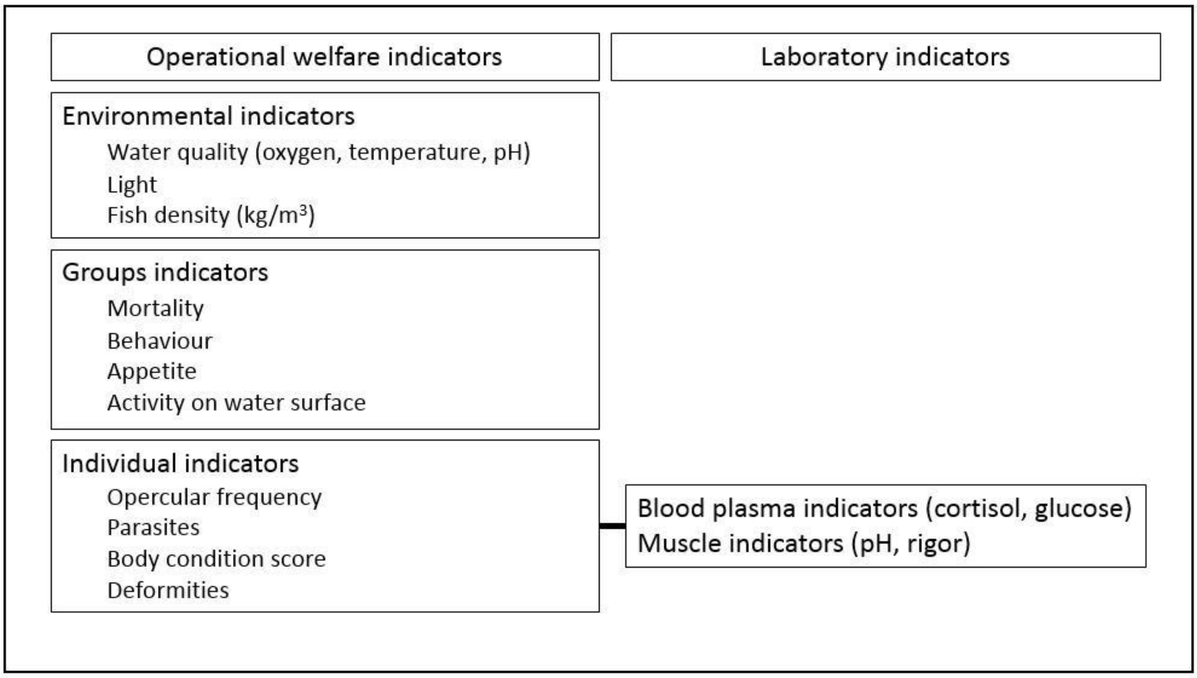


Figure 60: Summary of the operational indicators used on fish farms, including indicators that vary with the environment and the animal. The animal-based indicators can be based on groups of fish or on individuals, and individual indicators can include laboratory analyses.

In general, aquaculturists use feeding as an indirect indicator of welfare. That is, one approaches the tank and provides food, and the fish respond by going to the surface and eating, which is a good sign. If the fish do not come to eat, they have lost their appetite for some reason and more information is needed. Although there is plenty of equipment that can be purchased to feed fish automatically, it is recommended to feed fish at least once a day by hand in order to get an idea about how they are doing. If the fish do not eat, that will affect their weight gain, which is also relatively easy to measure. Another operational indicator that is common in fish farms is the coefficient of condition in live weight (the live weight divided by the cube of the length). It indicates nutritional status (Bavčević et al. 2010), and gives an idea about the amount of intraperitoneal fat. The hepato-somatic index (HSI) is defined as the ratio between the weight of the liver and the live weight. During periods of fasting, the needs for energy are met mostly by mobilising glycogen reserves from the liver, while the fat reserves are left more or less untouched during the first few days (Peres et al. 2014). Thus, HSI can be used to indicate energy reserves since the liver is an important regulator of nutrient use in fish (Christiansen & Klungsøyr 1987).

3 References

This document relating fish and aquaculture system description is fully based on the aqu@teach textbook (Jungle et al. 2020). The complete textbook is available at http://doi.org/10.5281/zenodo.3948179

Ashley, P.J. 2007. Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science 104 (3-4), 199-235.
Avnimelech, Y. 2014. Biofloc Technology – A Practical Guide Book (3rd edition). The World Aquaculture Society, Baton Rouge.
Bavčević, L., Klanjšček, T., Karamarko, V., Aničić, I. & Legović, T. 2010. Compensatory growth in gilthead sea bream (Sparus aurata) compensates weight, but not length. Aquaculture 301 (1-4), 57-63.
Bhilave, M.P., Nadaf, S.B. & Deshpande, Y.V. 2014. Proximate analysis of formulated feed. Originally published in 2010 in *All About Feed* 1(9).
Braithwaite, V. 2010. Do Fish Feel Pain? Oxford University Press, Oxford.
Canada Department of Fisheries and Oceans 2004. Animal User Training Template. 1.0. Anatomy and Physiology of Salmonids.
Castanheira, M.F., Conceicão, L.E., Millot, S., Rey, S., Bégout, M.L., Damsgård, B., Kristiansen, T., Höglund, E., Øverli, Ø. & Martins, C.I.M. 2017. Coping styles in farmed fish: consequences for aquaculture. Reviews in Aquaculture 9, 23-41.
Christiansen, D.C. & Klungsøyr, L. 1987. Metabolic utilization of nutrients and the effects of insulin in fish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 88 (3), 701-711.
Conte, F.S. 2004. Stress and the welfare of cultured fish. Applied Animal Behaviour Science 86 (3-4), 205-23.
Council Regulation (EC) No 2406/96 of 26 November 1996 laying down common marketing standards for certain fishery products.
Davidson, J., Good, C., Welsh, C. & Summerfelt, S.T. 2011. Abnormal swimming behavior and increased deformities in rainbow trout Oncorhynchus mykiss cultured in low exchange water recirculating aquaculture systems. Aquacultural Engineering 45 (3), 109-117.
Dawkins, M.S. 2017. Animal welfare with and without consciousness. Journal of Zoology, 301 (1), 1-10.
Hoar, W.S. & Randall, D.J. 1984. Fish Physiology: Gills: Part B – Ion and Water Transfer. Academic Press, Cambridge MA.
Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P. & Turnbull, J.F. 2006. Current issues in fish welfare. Journal of Fish Biology 68 (2), 332-72.
Junge, R., Antenen, N., Villarroel, M., Griessler Bulc, T., Ovca, A., & Milliken, S.(Eds.) (2020). Aquaponics Textbook for Higher Education. Zenodo. http://doi.org/10.5281/zenodo.3948179
Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X. & Semmens K. 2014. An international survey of aquaponics practitioners. PloS One 9, e102662.
Moffitt, C.M. & Cajas-Cano, L. 2014. Blue Growth: the 2014 FAO state of world fisheries and aquaculture. Fisheries 39 (11), 552-553.
Mozanzadeh, M., Marammazi, J.G., Yaghoubi, M., Yavari, V., Agh, N. & Gisbert, E. 2017. Somatic and physiological responses to cyclic fasting and re-feeding periods in sobaity sea bream (Sparidentex hasta, Valenciennes 1830). Aquaculture Nutrition 23, 181-191.
Noble, C., Gismervik, K., Iversen, M.H., Kolarevic, J., Nilsson, J., Stien, L.H. & Turnbull, J.F. (eds.) 2018.
Welfare indicators for farmed Atlantic salmon: tools for assessing fish welfare.
Nordgreen, J., Garner, J.P., Janczak, A.M., Ranheim, B., Muir, W.M. & Horsberg, T.E. 2009. Thermonociception in fish: effects of two different doses of morphine on thermal threshold and post-test behaviour in goldfish (Carassius auratus). Applied Animal Behaviour Science 119 (1-2), 101-107.
Okawara, Y., Ko, D., Morley, S.D., Richter, D. & Lederis, K.P. 1992. In situ hybridization of corticotropin-releasing factor-encoding messenger RNA in the hypothalamus of the white sucker, Catostomus commersoni. Cell and Tissue Research 267 (3), 545-549.
Peres, H., Santos, S. & Oliva-Teles, A. 2014. Blood chemistry profile as indicator of nutritional status in European seabass (Dicentrarchus labrax). Fish Physiology and Biochemistry 40 (5), 1339-1347.
Pickering, A. & Pottinger, T. 1995. Biochemical effects of stress. Biochemistry and Molecular Biology of Fishes 5, 349-379.
Reimchen, T.E. & Temple, N.F. 2004. Hydrodynamic and phylogenetic aspects of the adipose fin in fishes. Canadian Journal of Zoology 82, 910-916.
Rose, J.D. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science 10 (1), 1-38.
Sneddon, L.U. 2003. The evidence for pain in fish: the use of morphine as an analgesic. Applied Animal Behaviour Science 83 (2), 153-162.
Sumpter, J., Le Bail, P., Pickering, A., Pottinger, T. & Carragher, J. 1991. The effect of starvation on growth and plasma growth hormone concentrations of rainbow trout, Oncorhynchus mykiss. General and Comparative Endocrinology 83 (1), 94-102.
Villarroel, M., Junge, R., Komives, T., König, B., Plaza, I., Bittsánszky, A., & Joly, A. 2016. Survey of aquaponics in Europe. Water 8 (10), 468.
Wainwright, D.K. & Lauder, G.V. 2017. Mucus matters: The slippery and complex surfaces of fish. In Gorb, S.N. & Gorb, E.V. (eds.) Functional Surfaces in Biology III: Diversity of the Physical Phenomena, pp. 223-246. Springer, New York.
Zupanc, G.K. 2009. Towards brain repair: insights from teleost fish. Seminars in Cell and Developmental Biology 20 (6), 683-690.
Boyd, E. (2018). Water temperature in aquaculture. Global Aquaculture Advocate.
Timmons, M.B., Ebeling, J.M. (2010). Recirculating Aquaculture. 2nd Edition. Cayuga Aqua Ventures, Ithaca, NY.
Bregnballe, J. (2015). A Guide to Recirculation Aquaculture – An introduction to the new environmentally friendly and highly productive closed fish farming systems. The Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation.
Gomon, Martin; Bray, Dianne. "Rainbow Trout, *Oncorhynchus mykiss*". *Fishes of Australia*. Retrieved August 30, 2014
FAO 2022. Cyprinus carpio. Cultured Aquatic Species Information Programme. Text by Peteri, A.. Fisheries and Aquaculture Division [online]. Rome. Updated 2005-07-12

FAO 2018a. *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.* Licence: CC BY-NC-SA 3.0 IGO.
FAO 2018b. Aquaculture Feed and Fertilizer Resources Information System. Rainbow trout. Food and Agriculture Organization of the United Nations.
Fitzimmons, K. 2018. Introduction to tilapia nutrition. University of Arizona.
FAO 2022. *Oreochromis niloticus. Cultured Aquatic Species Information Programme. Text by Rakocy, J. E.. Fisheries and Aquaculture Division [online]. Rome. Updated 2006-05-19*
Gibbens, S. 2019. Climate change is depleting our essential fisheries. *National Geographic* online. Retrieved 15. February 2019.
Harper, C. & Wolf, J.C. 2009. Morphologic effects of the stress response in fish. *ILAR* 50 (4), 387-396.
Lazzarotto, V., Médale, F., Larroquet, L., & Corraze, G. 2018. Long-term dietary replacement of fishmeal and fish oil in diets for rainbow trout (Oncorhynchus mykiss): Effects on growth, whole body fatty acids and intestinal and hepatic gene expression. *PloS One*, 13(1), e0190730.
Oliva-Teles, A. 2012. Nutrition and health of aquaculture fish. *Journal of Fish Diseases* 35 (2), 83-108.
Polanco, J. F. & Bjorndal, T. 2018. Paper 2 Aquaculture diversification in Europe: the kingdom of Spain and the kingdom of Norway. In *Planning for aquaculture diversification: the importance of climate change and other drivers: FAO Technical Workshop 23–25 June 2016, Rome Italy* (p. 37). Food & Agriculture Org.
Seawright, D.E., Stickney, R.R. & Walker, R.B. 1998. Nutrient dynamics in integrated aquaculture-hydroponics systems. *Aquaculture* 160, 215-237.
Somerville et al. 2014. Introduction to aquaponics. In Somerville, C., Cohen, M., Pantanella, E., Stankus, A. & Lovatelli, A. *Small-scale Aquaponic Food Production: Integrated Fish and Plant Farming*. FAO Fisheries and Aquaculture Technical Paper No. 589. Food and Agriculture Organization of the United Nations, Rome, pp. 1-10.
Timmons, M.B. & Ebeling, J.M. 2007. Recirculating Systems. Northeastern Regional Aquaculture Center, Ithaca, NY.
Timmons, M.B., Riley, J., Brune, D., and Lekang, O.-I. 1999. Facilities Design. In CIGR–The International Commission of Agricultural Engineering (eds.) *CIGR Handbook of Agricultural Engineering Volume II: Animal Production & Aquacultural Engineering*. *American* Society of Agricultural Engineers, USA. pp 245-280.
Tran-Ngoc, K. T., Dinh, N. T., Nguyen, T. H., Roem, A. J., Schrama, J. W., & Verreth, J. A. 2016. Interaction between dissolved oxygen concentration and diet composition on growth, digestibility and intestinal health of Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 462, 101-108.
Tschudi, F. 2018. *Planning basis for dimensioning the aquaculture part of an aquaponic system.* Wädenswil: Zurich University of Applied Sciences, Research Group Aquac