PathoGelTrap

New Blue Revolution through a pioneering pathogen-trapping technology based on bioselective hydrogel-forming proteins

H2020 – FET OPEN - Challenging Current Thinking

Deliverable No. 3.4

Assessment of most common filtrations solutions adopted by aquaculture industry

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Executive Summary

Among the objectives of WP3 of the PathoGelTrap project, task 3.4 assesses the most common water filtration systems used by fish farms. A considerable difference was found between Freshwater and Marine water farms in the results of a survey on the use of filtration systems. Concerning mechanical filtration systems of freshwater farms, 30% use drum filters, 30% use sand filters, and 20% use grid filters. The rest of the farms do not use any kind of mechanical filtration. Among the marine farms, 81% of them use sand filters. 33% of these combine them with grid and drum filters and 22% together with drum and cartridge filters.

Relevant characteristics for PGT filter design of the existing filtration systems in the industry have been considered: Operating pressure, Flow through filtering media, Material of the filtering media, and Unit size of the filtering material. It is possible that other characteristics are found relevant as the project continues to develop, and they will be used for filter concept design

These results will be used in Task 3.2 to develop the PGT filter.

List of acronyms/abbreviations

AFB= Affibodies
DL= Deliverable
EC= European Commission
EU= European Union
IZSVE= Istituto Zooprofilattico Sperimentale Delle Venezie
LCRs= Low Complexity Regions
LLPS = Liquid-Liquid Phase Separation proteins
LLPS-AFB chimera= Chimeric biomimetic material
PGT= PathoGelTrap
SW: Smartwater Planet S.L.
VNNv= Viral Nervous Necrosis virus
VLP= Virus Like Particle
WP= Work-Package
YR= *Yersinia ruckeri*

Glossary of terms

Term	Explanation
rcSso7d binder	Reduced charged Sso7d protein (rcSso7d), from the hyperthermophylic archaeon Sulfolobus solfataricusis. This protein is an attractive binding scaffold that has been designed for biotechnological applications (AFBs more for biomedical applications), being more thermostable and robust than AFBs. Furthermore, it has a small size of 64 aa (7 kDa) (AFB: 6.5 kDa) and 9 variable positions (AFB: 13). The binding site is located on the surface of a rigid β-sheet, which is also an advantage in comparison to other alternatives binders.
Affibody	Small engineered binding protein based on a three-helix bundle motif of the Z domain derived from staphylococcal protein a. All affibodies contain 58 residues where variations affect to 13 positions of helix 1 and 2.
Betanodavirus	Viral agent causative of Viral Nervous Necrosis, also known as viral encephalopathy and retinopathy, one of the target infective agents of the PGT project
Yersinia ruckeri	Bacterial agent causing Enteric Redmouth disease, one of the target infective agents of thePGT project
Pathogen	Organism able to cause disease

Antigen	Any substance that causes the immune system to produce antibodies against itself. In terms of pathogens, antigens are a protein (or part of it) exposed on its surface and capable of being recognized.
Liquid-liquid phase separation (LLPS)	Certain molecules (such as proteins) are rearranged into a dense phase that coexists with a dilute phase reminding liquid droplets.

1. Introduction

PathoGelTrap aims to transform the aquaculture future through a paradigm change in infectious-disease management practices by providing the industry with a pioneering pathogen-trapping technology able to target and remove specific pathogens directly from the water. Going way beyond the state of the art, *PathoGelTrap*'s Consortium will use the current knowledge on self-assembling properties of the Liquid-Liquid Phase Separation proteins (LLPS) to rationally design a biomimetic material that will efficiently recognize and trap fish pathogens (both viruses and bacteria) directly in the water and inactivate them.

Thanks to the versatility offered by LLPS proteins, we propose to provide the industry with two different solutions, PathoGelTrap Liquid and PathoGelTrap Filter. The final objective of the PGT project is to have operational products for the treatment of rearing water that remove the target pathogens from the water. Hence, it is important that the criteria needed for these products are fed into the models from the start.

The objective of this deliverable is to assess the most common filtration systems currently used in the aquaculture industry to help with the design of the PGT filter.

2. Water filtration systems

2.1. Filtering/water treatment systems in aquaculture operations

Typically, water filtering/treatment systems are associated to on-land operations, *i.e.* hatcheries, nurseries or RAS (Recirculation Aquaculture System) units devoted to any of the above or, lately, for on-growing to market size.

These treatments are used to deal with suspended solids (mechanical filters), microbial/live particles in the water (disinfecting treatments), or dissolved chemicals or metabolites (biofilters, oxidative chemical treatments, foam fractionators or skimmers).

2.1.1. Mechanical filters

Mechanical filters remove suspended solids (organic or inorganic) from the water down to a nominal filtration value, frequently expressed in microns. They are the first barrier of filtration and can be sequenced to match the requirements of different phases of production, according to the sensitivity of the live phase to cater for (eggs, larvae, fry, nursery or adult fish) or even the needs of live feed production. The removal of suspended solids particles can bring with it the removal of microorganisms associated to these particles as substrate. This is a side effect but, even if it is not the main intention of the equipment as such, it is not a minor one.

2.1.1.1. Grids or screens

Most farms have some kind of screen or grid at the point of water intake to avoid the entrance of big materials like leaves, branches, etc. They are "passive" filters, non-pressurized, typically placed at the suction end of the intake pipe, or at the entrance of a pumping station.

2.1.1.2. Sand filters

These are also used in many farms to get rid of most of the suspended solids at the main water intake pipes. Typically, they are balloon-like or cylindrical containers filled up with sand (silica sand), frequently several of them in a parallel or serial arrangement. Most frequently, water is pumped through them, pressurized. The size of the sand grains and the speed of the flow through the filters (related to the total filter volume and modulated by the filter´s arrangement mentioned above) determines the filtering efficiency and the particle size being removed. Efficient sand filtering arrangements can filter particles down to 60 microns or less. They need frequent cleaning of the sand bed (by back-flushing) to avoid clogging, or the formation of preferential channels through the sand, or even the establishment of microbial communities which could even translate to anoxic conditions inside the sand bed, flushing dangerous metabolites into the water. Sand filters can also be used at different points inside the farm for smaller water supplies or as part of the water treatment system for solids removal in RAS. Their advantages are, mainly, that they are cheap, fairly reliable, easy to operate and have a low maintenance cost. For these reasons, they are extensively used in fish farms.

2.1.1.3. Drum filters

Drum filters are very much in use in RAS units and also in freshwater operations for the filtering and re-utilization of water, and at times for the removal of solids before effluent release. They consist of a rotating drum, lined with a screen mesh. The water enters the inside of the drum and goes out through the mesh, where the solids are retained. There is an arrangement of nozzles that clean the mesh, gathering the solids sludge in an evacuating channel and conducting it to a secondary treatment or to a concentrating system. Mesh sizes are normally around 35 microns for standard application. They are not pressurized and they remove the suspended solids avoiding, in the most part, breakage of the particles into smaller ones that would be more difficult to remove from the water. Disc filters are similar but, instead of the mesh being configured as a drum, several rotating discs parallel to the water flow act as the filtering structure.

2.1.1.4. Cartridge filters

When the production phase requires greater water quality, cartridge filters are used to filter out particles below 10-5 microns (larvae or small fry), even 1 or 0,5 microns (live feed production, egg incubation, yolk sac stage larvae). This level of solid filtration is normally coupled with disinfection treatments, to increase their efficiency.

2.1.2. Disinfecting treatments

Disinfecting treatments are very specific treatments for biosecurity applications. Biosecurity is gaining more importance in recent years in aquaculture operations. On land operations ("pump-ashore operations") allow having major biosecurity controls, and this is gaining more consideration as the sector matures and the business/investment security requirements increase. Disinfecting treatments were only the exception some 15 years ago, but now at least some form of disinfecting treatment is commonplace in any production unit (unless it has the advantage of a bore-hole water supply). They are not cheap treatments, so they are associated with valuable crops or operations (marine seabass and seabream, big salmon operations).

2.1.2.1. Ultraviolet Light Treatment

Ultraviolet Light Treatment (UV treatment) or UV filtration, is the most commonly used water disinfecting treatment. As mentioned above, it is usually coupled with suspended solids removal to increase its efficiency. They can be configured as pressurized systems (intersected in the pipes) or in open channels. It can be found in two different applications: at the main water supply, to secure the treatment of all the water coming into the farm; or within particular parts of the operation. Again, the most sensitive stages of the production cycle (live feeds, egg incubation, yolk sac larvae, initial stages of larval development) would be the target for this treatment. UV intensity sensitivity is a characteristic of the different pathogens, viral or bacterial, and the choice of the intensity of the equipment is a major exercise, as there is a trade-off between the cost (investment and operational energy consumption) and the safety of the crops. Hatcheries are big users of UV treatment, not only for the biosecurity of their production cycles but also for the sanitary guarantee towards the clients. RAS operations are also big users of UV in the systems water treatment design, as it is a way to control or modulate the microbial levels within the system´s water.

2.1.2.1. Ozone Treatment

Ozone is a very potent oxidant that has been used to totally disinfect (sterilize) the main water supply entering some farms, particularly in Greece, during the early 2000s. However, it is very dangerous as it is poisonous for fish as well as the microbes (and humans). Dosing levels are controlled, among other means, by the use of “Red-Ox Potential” reading probes, which are, still today, not that reliable in operation. Safety measures have to be taken, like activated carbon filtration of all the water post ozone treatment, to make sure all O_3 is removed from it before contacting the fish, as well as by-products from the ozone chemical interaction with the seawater chemistry. A somewhat less troublesome application is the dissolution of ozone in the water to non-sterilizing levels, keeping moderate Red-Ox readings (around 350 mV) in RAS units. The contact of the ozone with the water is typically achieved by the use of skimmers (see below) as contactor containers, which build up the benefits of both. This keeps a “healthy” lower microbial count, contributes to breaking down colouring dissolved organics (typical “coffee colour” of any RAS water), and creates a better environment in the water. Ozone treatment systems are safer now and more frequent than they used to be, due to the improvements in the control and the hazards associated with them.

2.1.3. Chemical filtration

Chemical filtration, using “chemical” in a very loose manner, mainly deals with the removal of metabolites or other chemicals in the water. It is considered here as an eclectic classification concept as it collates very diverse physic-chemical processes that can have heterogeneous effects, as it can be understood from the descriptions of the different methods.

2.1.3.1. Biofilters

Every RAS has one. In essence, it consists of a chamber (submersed or trickling) with a settling material for a biofilm to grow on, through which the water from the fish tanks in the RAS flows. The settling material or bio-filtration material serves the purpose of multiplying the available surface for the development of the biofilm in a given volume. It can be submerged in the water to be treated or the water can be percolated from the top of the biofilter chamber on the bio-filtration material, favouring the contact of water and air and aiding on the oxygenation of the water. A third configuration has the biomaterial moving in the water column inside the contact chamber (moving bed biofilter). Within the biofilm, different communities of bacteria grow and consume (apart from oxygen) the nitrogenated products excreted by the fish, turning harmful ammonia into less harmful nitrites and these to almost carefree nitrates. Although a biofilter does not destroy bacteria, the microbiome that settles in the system is so varied and large that a prospective pathogen has to compete to become an outburst of pathology. A healthy biofilter can act as a buffer in certain cases, and the application of un-specific disinfection treatments within the RAS has to be managed accordingly.

2.1.3.2. Electro-oxidation

Electro-oxidation is a relatively new development, although it has taken different forms in recent years, and has not really taken on yet. Still, it is worth mentioning, as it has several applications and benefits. Electro-oxidation works, like ozonation, by forming very highly oxidizing and moderately short-lived chemical species in seawater (mostly Cl-), in a very reduced “electrical chamber”. This not only oxidizes microbial particles (bacterial and viral) to sterilization, but also breaks metabolites like ammonium. This means that one treatment can disinfect and get rid of the nitrogen metabolites completely from RAS´s water. The technology is still in development but might come up to cost-effectiveness soon.

2.1.3.3. Skimmer

A skimmer works on the principle of surfactants and it is gaining popularity in its use in marine aquaculture facilities for its versatility (the principle does not work so efficiently in fresh water), particularly as part of RAS. They consist of a tall (up to 5 meters or more) cylinder where water is pumped in and ascends up to maybe a fifth of the top, where the outlet is. Air or oxygen is injected at the bottom of the cylinder in very small bubbles. On their way up these bubbles collect and form micelles with polar particles in the water, which gather up as foam that floats in the surface (above the outlet) and is channelled upwards by its own consistency through to a collecting chamber on top of the skimmer. This forming foam can collect many different particles, from small suspended solids (down to small clay particles), to bacteria, even large organic molecules or viruses, removing them from the water. To make this process even more efficient, the water outlet normally connects with a downward pipe within the skimmer, below the water inlet, so that the water has to go against the current of bubbles to come out. At the same time, water is oxygenated and, as mentioned above, air can be substituted with ozone as its contacting chamber, multiplying the efficiency of both.

2.2. Assessment of Filtering/water treatment systems

Table 1 shows different characteristics of filtration systems as regularly used in aquaculture. We have considered Operating pressure, Flow through filtering media, Material of the filtering media, and Unit size of the filtering material. These are considered relevant characteristics from what we currently know about the stability and properties of the hydrogels the project is developing. It is possible that other characteristics are found relevant as the project continues to develop, and they will be used for filter concept design (Task 3.2).

Table 1: Relevant characteristics of different filtering systems applied in aquaculture

	Operating pressure (bar)	Flow (m3/m2/h)	Material of filtering media	Filtering media unit size (mm)
Grid filter	0		Stainless steel	-
Pressurized sand filter	2-5	9-30	silica	0,5-1,2
Drum filters	0			
Cartridge filters	10 bar structural < 1 bar effective*	0,6-1	Polypropylene, PTFE, cellulose, nylon, stainless steel	
U.V.	Low-High	-	no media	-
Ozone	0	-	no media	-
Biofilters:				
Percolating biofilters	0	0,1-0,4	Shaped Polyetilene, Polypropylene, ceramics	15-30**
Submerged biofilters	0	0,1-0,4	Shaped Polyetilene, Polypropylene, ceramics	15-30**
Moving bed biofilters	0	0,1-0,4	Shaped Polyetilene, Polypropylene, ceramics	10-20**
Electro-oxidation	-	-	no media	-
Skimmers	-	-	no media	-

*Effective pressure through the material.

** Media is shaped in different ways to increase surface to volume rate.

Hydrogels are delicate structures and could be affected by the strength of the flow through the PGT filter. Also, the 3D structure of the hydrogel is a challenge to achieve an efficient circulation of the water to ensure the contact of the pathogenic particles with the binder, which promotes their capture and removal from water. Possible recovery of the functionality of the filter is another challenge. Materials and configuration have to allow for treatment to recover functionality. The durability of the PGT filter, to be able to install it and use it for a significant amount of time with continuous functionality. The active life of the product in the filter should be compatible with economic cost efficiency even when no pathogen is present.

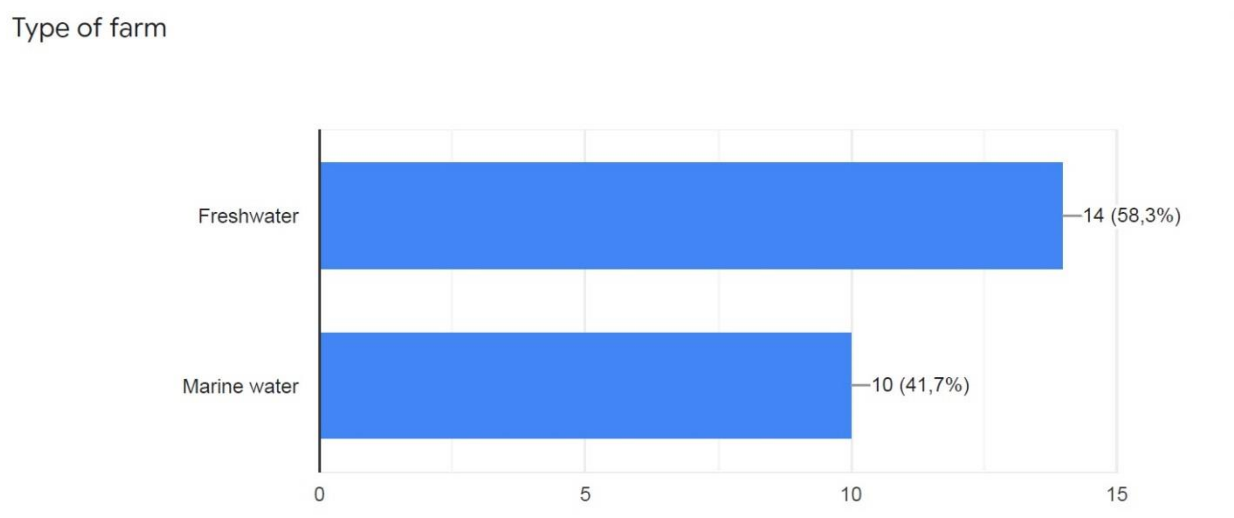
Technical aspects will be considered during the conceptual and technical design in Task 3.2

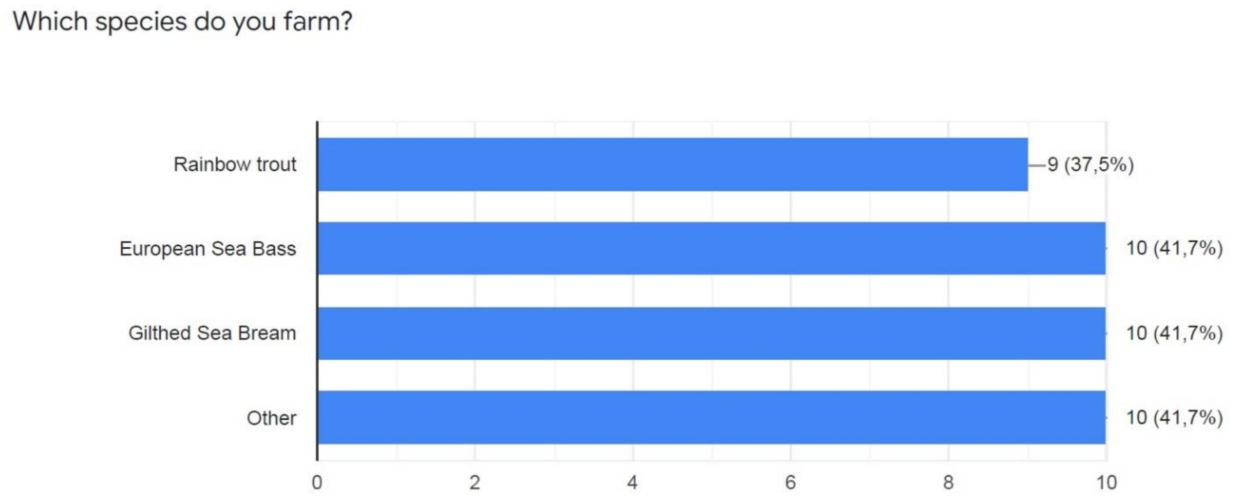
3. Survey on most common filtration systems

A survey was designed (on Google Forms) and sent individually to 70 relevant European operators (farmers and vets/biologists involved in aquaculture) to be filled. We have received answers from 24 of these and plan to make the survey available for 2 further months. Moreover, the form is available online on PGT Social Media (https://lnkd.in/dufWr_m).

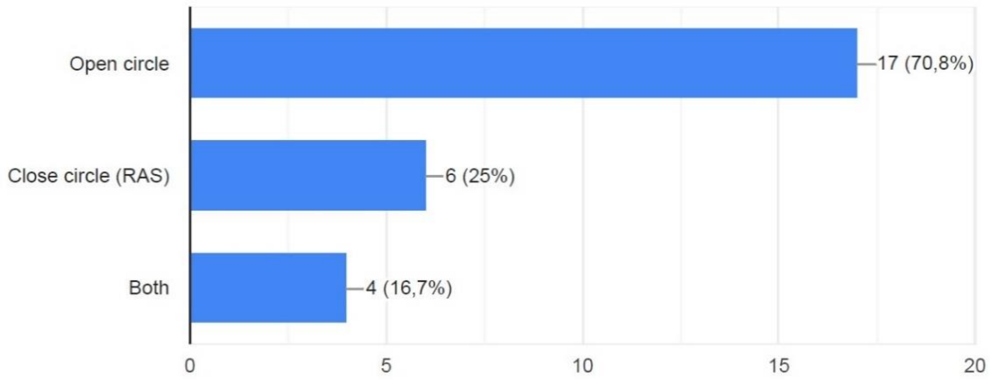
3.1. Survey results

The results from the "*Survey on water filtration systems*" are reported below:





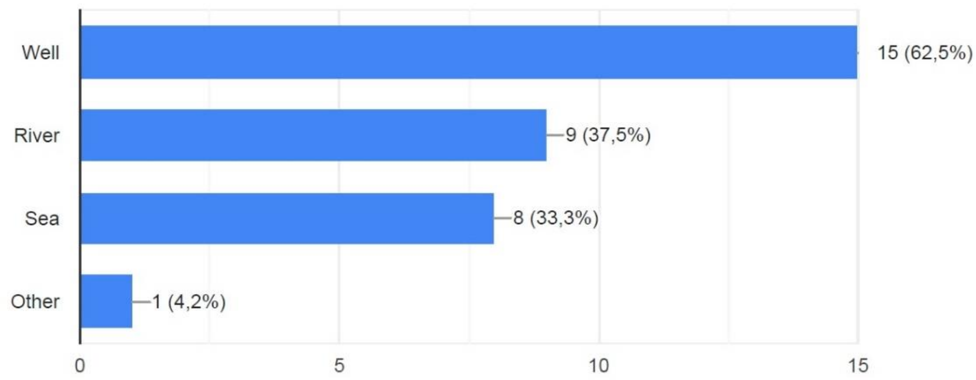
Which type of water system do you use?



If "both", please write the percentage of RAS used

- *15% of total holding volume is RAS 80%*
- *15% new water*
- *25%*
- *50% Broodstock on RAS, 50% on Open flow. Some pregrowing with partial RAS*

Which kind of water supply do you use?



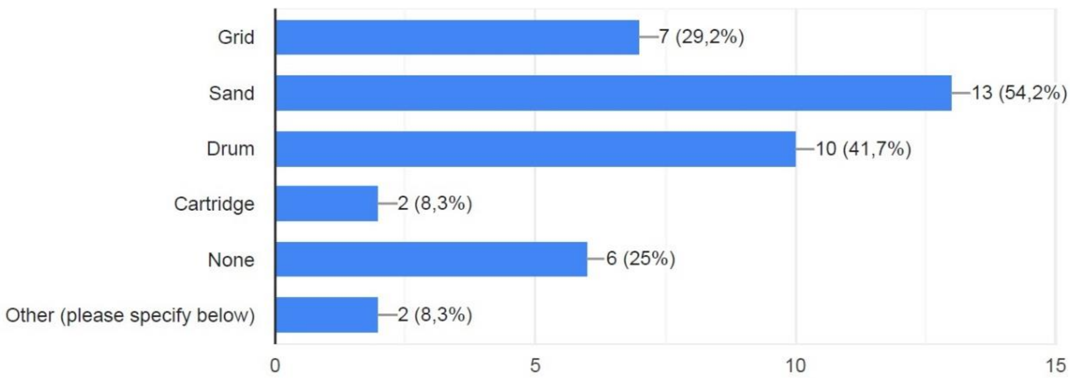
How many liters do you filter daily?

- *450.000*
- *400*
- *40.000.000*
- *1000 -1500 cubic meter/hr*
- *8.640.000*
- *150 cubic meter per hour= 3600 m^3/day*
- *500-1100 m^3 depending on season*
- *450.000*
- *45.000.000*
- *50 cubic meters*

- *5000*
- *900000*
- *500-800*

What kind of filter do you have in inlet water?

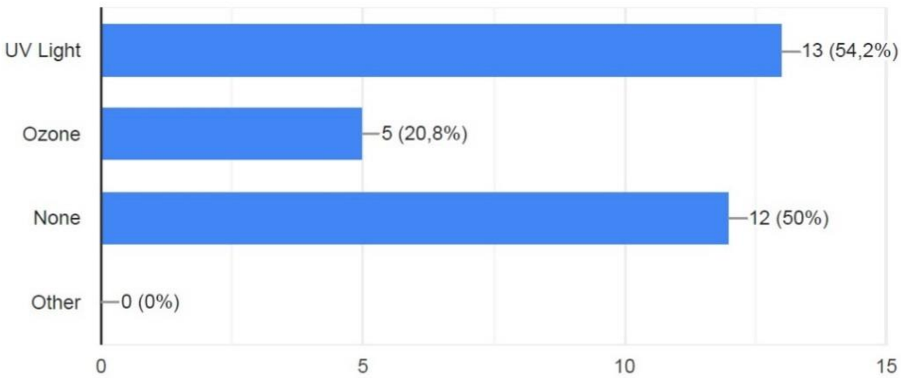
Mechanical filters:



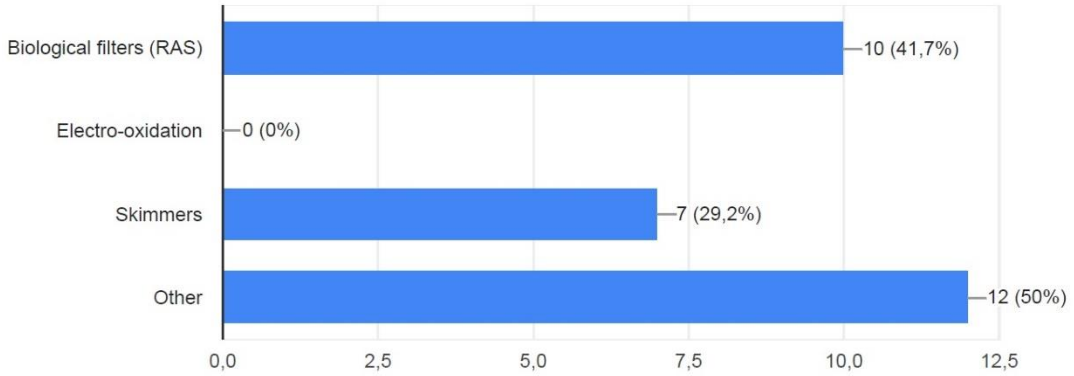
If "Other", please specify

- *Settling pond*
- *Pre-filtration by rings*

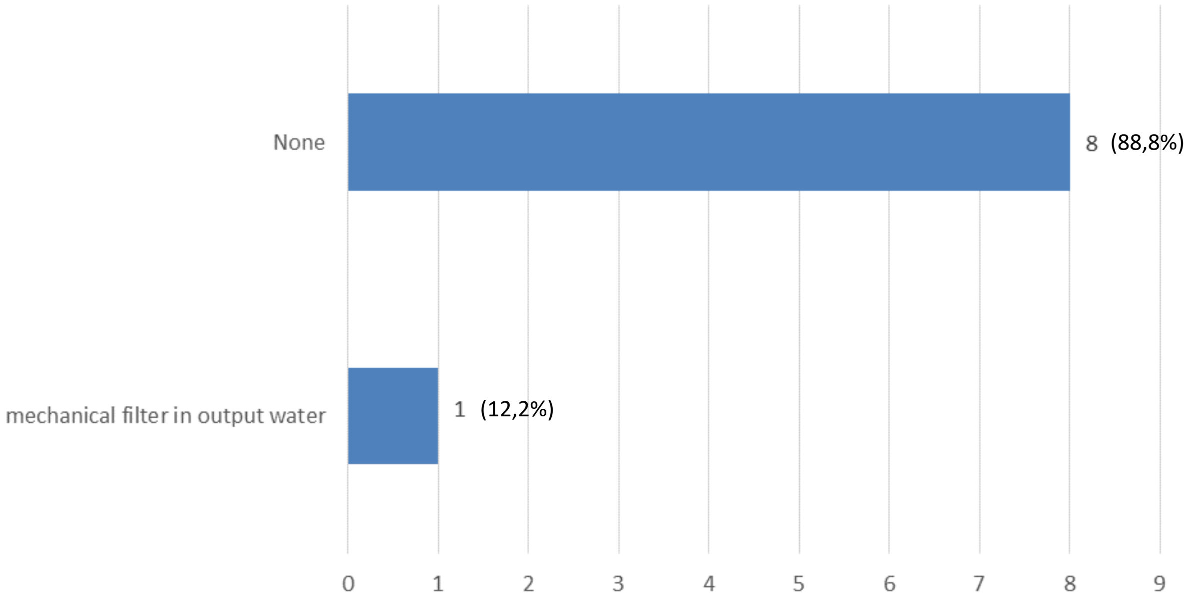
Disinfecting treatment:



Other filters:



If "Other", please specify



This information will be used for the design of the filtration system to be used in Task 3.2.

3.2. Discussion of the survey results

This discussion is referred only to the data obtained from the responses of the survey (24 farms). The survey will be kept open through the next 2 months and results will be revised conveniently.

When considering the type of farm separately, the results showed that for Freshwater farms rearing rainbow trout all the farms use flow-through water system, 22% takes water only from river, where the rest (78%) takes it both from river and well.

Concerning mechanical filtration systems, 30% use drum filters, 30% use sand filters and 20% use grid filters. The rest of the farms do not use any kind of mechanical filtration. Only one farm uses settling ponds instead of the other options.

Concerning disinfection treatments, most of them (55.5%) do not use any disinfection system, while the rest (44.5%) use UV light.

Finally, they don't use any other filter and the mean flow of water filtered is around 10.000 m^3/day on average.

If considering Marine water farms, 27% of them use only RAS, 45% use only flow-through, and the rest (27%) use both RAS and flow-through. In the latter case, 50% of the water is recirculating (on average), while the rest is new water. The water supply is for 54% of the farms directly from the sea, 27% only from well and the rest (19%) from both well and sea. The flow of water filtered is around 20.000 m^3/day on average.

Concerning mechanical filtration systems, 81% of the farms use sand and 19% do not use any kind of filter. Concerning sand filters, 25% use it alone, 33% of farms use it in combination with grid and drum filters, 22% together with drum and cartridge filters, 10% with grid, and 10% with drum filters. The presence or not of RAS does not affect the results.

Concerning disinfection treatments, most of the farms (72%) use UV Light, 62% of them together with Ozone, while 18% do not use any disinfection treatment.

Biological filters are used by all farms that have a RAS system (54%), also Skimmers are widely used (64%).

In conclusion, for Freshwater farms, drum and sand filters are equally used, while no biological filters are involved. Concerning Marine farms, sand filters are the most used, often together with grid and drum filters. Biological filters are widely used, because most of the farms use RAS.

This information will be used for setting the filtration system to be used in Task 3.2.

3. Overall monitoring and evaluation of results

Filtering systems common in the aquaculture industry have been identified and assessed with regard to relevant characteristics to the PGT filter design.

The effort will be maintained to consider new relevant characteristics as the project develops more knowledge about the properties of the PGT chimera and hydrogels forming.

The response to the questionnaire was satisfactory, and the answers are well represented both for freshwater and water, as well as for the fish species. It appears that the most commonly used filters are drum and sand, together with biological filters when RAS is involved.

We will keep the survey online for 2 additional months, before concluding, and feed any new results as relevant information for Task 3.2.

4. Conclusion

Relevant characteristics for PGT filter design of the existing filtration systems in the industry have been considered: Operating pressure, Flow through filtering media, Material of the filtering media, and Unit size of the filtering material.

It is possible that other characteristics are found relevant as the project continues to develop, and they will be used for filter concept design. These results will be used in Task 3.2 to develop the PGT filter.