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## Ultrafiltration for the biosecurity of fish production: the case of a sturgeon nursery

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**Abstract:** Sturgeon farming requires special attention. In addition to a relatively long rearing, climate change has resulted in increasingly high temperatures favorable to the emergence of pathogens. The control of water quality is essential especially the first years of life of the fish to prevent a mimivirus (AcIV-E) and a mycobacterium (*Mycobacterium liflandii*). These crises can lead to significant mortality (up to 70%) and have only been documented in hatchery populations where mortality can reach as high as 100%. *Mycobacterium liflandii*, fatally affects young generations of Siberian sturgeon (*Acipenser baerii*) mainly in year N+1 with mortalities reaching 30% when river temperatures rise above 21°C (summer period). The retention of these pathogenic microorganisms and of total flora by ultrafiltration was evaluated at a semi-industrial scale. The first part focuses on the specific removal of mimivirus, total flora and mycobacteria and the second part aims to evaluate over the long term (5 months) the hydraulic performances of the ultrafiltration process. Although the temperature (maximum 21°C) was not sufficient for the mycobacteria to be detected, a good retention of mimiviruses (up to 4.7 log removal) and total flora was observed throughout the study. A more stable water quality was obtained after ultrafiltration and this work confirms the potential great interest of ultrafiltration for the biosecurity of fish production.

**Keywords:** ultrafiltration, fish farming, biosecurity, water reuse, mimivirus

### Highlights:

- Ultrafiltration enables up to 4.7 log removal rate of mimivirus AcIV-E in water
- Ultrafiltration retains total flora from 2 to 5 log removal rate in water
- Ultrafiltration provides a more stable quality of water for sturgeon rearing
- Ultrafiltration hydraulic performances were stable over 5 months of operation

## 1. Introduction

The significant decline in natural sturgeon populations during the 20<sup>th</sup> century led to a worldwide ban on fishing for this fish. As a result, the production of these fish in fish farms, which are sought after for their roe and flesh, is booming (Bronzi *et al.*, 2011). However, intensive production may expose the fish to infectious diseases caused by pathogenic bacteria and/or viruses (Radosavljević *et al.*, 2019). Sturgeon, and particularly oscietra (Russian sturgeon - *Acipenser gueldenstaedtii*), are confronted with a mimivirus (AcIV-E) with a size of about 0.2-0.3  $\mu\text{m}$  capable of causing significant mortality of 30% to 70% in a few days (Clouthier *et al.*, 2020; Stachnik *et al.*, 2021). Outbreaks of mimivirus disease resulting in mass mortalities in sturgeon populations were first described in White Sturgeon *Acipenser transmontanus* in California (Hedrick *et al.*, 1990). Sturgeon mimivirus epidemics typically occur during the juvenile life stage (< 1 yr) of sturgeon and have only been documented in hatchery populations where mortality can reach as high as 100%. At least nine sturgeon species in the genera *Acipenser* or *Scaphirhynchus* of the family *Acipenseridae* are vulnerable to a mimivirus infection (Bigarré *et al.*, 2017; Ciulli *et al.*, 2016; Clouthier *et al.*, 2015, 2013; Hedrick *et al.*, 1990; Hofsoe-Oppermann *et al.*, 2020; Mugetti *et al.*, 2020). In addition to the impact on aquaculture production, these contamination episodes could also threaten the environment through discharge fish production effluents containing pathogens into the natural water bodies. Outbreaks of viral disease, particularly those associated with mimiviruses, pose a potential risk for strategies designed to aid the recovery of threatened sturgeon populations worldwide (Axén *et al.*, 2018; Bigarré *et al.*, 2017; Ciulli *et al.*, 2016; Doszpoly *et al.*, 2017; Mugetti *et al.*, 2020). In addition, the Isle basin, in Southern France, has been affected for several years by *Mycobacterium liflandii*, which fatally affects young generations of Siberian sturgeon (*Acipenser baerii*) mainly in age N+1 with mortalities ranging from 5% to 30% when river temperatures rise above 21-22°C. It can be detected by microscopic observation of internal organs but also by PCR (Polymerase Chain Reaction) technique on internal tissues (Fremont-Rahl *et al.*, 2011; Talaat *et al.*, 1997; Williamson *et al.*, 2008). The development of recirculating aquaculture system allows for effective control of water quality and disease by reusing the majority of the water after treatment within the system. In addition, the biosecurity of the low flow of renewal water is necessary to guarantee the biosecurity of production. In this context, ultrafiltration (UF), known for its performance in eliminating bacteria and viruses, could be a suitable process for both water treatment for reuse and water treatment of renewal water. Thus, the objective of this study was to develop the UF process for the treatment of river water entering a sturgeon nursery with the aim of integrating it into a recirculating system. UF performances in terms of water quality and removal of pathogenic microorganisms were assessed as well as its hydraulic performances towards a variable quality of inlet water.

## 2. Materials and Methods

### 2.1 UF membrane and pilot plant

The UF membrane used for the experiments were hollow fibers (ZeeWeed ZW700B, SUEZ, Aquasource, France) in polyethersulfone with 7 channels of 0.9 mm inside diameter and 1.2 m in length. Their molecular weight cut-off was 200 kDa with a pore size of about 0.02  $\mu\text{m}$  and initial pure water permeability ( $L_p$ ) at 20°C of 900 L h<sup>-1</sup> m<sup>-2</sup> bar<sup>-1</sup> with a 9 m<sup>2</sup> of membrane surface

(Cordier *et al.*, 2019b, 2019a, 2018). The semi-industrial pilot plant, able to treat up to  $20 \text{ m}^3 \text{ d}^{-1}$ , was deployed under real conditions in the fish nursery of a caviar production site in Neuville (France). The tests were all performed in dead-end filtration. Hydraulic performances, membrane permeability ( $L_p$ ) and transmembrane pressure (TMP), were registered every minute by the pilot system. Turbidity was measured and recorded every minute in the feed tank of the UF pilot plant using a prob VisoTurb 700 IQ (WTW, Xylem Analytics). Various cleaning operations were automatically carried out at defined frequencies to eliminate membrane fouling: backwashing and air coupled with chlorinated backwashing. UF permeate with 10 ppm  $\text{NaClO}$  was used as chlorinated water for backwashing. The frequency of backwash was 3 classic backwashes followed with 1 air and chlorinated backwash. The detailed information about these types of backwash can be found in Yang *et al.* (2021). Chemical cleanings were triggered when the permeability reached a limit of  $200 \text{ L h}^{-1} \text{ m}^{-2} \text{ bar}^{-1}$  or after 4 days without chemical cleaning. The following filtration conditions were defined following a preliminary study: filtration flux,  $J = 60 \text{ L h}^{-1} \text{ m}^{-2}$ , filtration time,  $t_f = 60 \text{ min}$  and a water recovery rate of around 98% is obtained (Yang *et al.*, 2021).

## 2.2. Feed water

The UF pilot was continuously fed with river water that had passed through sturgeon *Acipenser baerii* rearing tanks and was pre-treated by sand filtration. 125 kg of sand (0.3-0.6 mm) were used in the sand filter with a filtration pore size of around 40-60  $\mu\text{m}$ . Backwashes of the sand filter were operated manually when the pressure drop exceeded 1 bar. The aim of not treating the river water directly was (i) to increase the chances of having a higher concentration in microorganisms (ii) to simulate a recirculated process by passing through the pre-growth tanks (Figure 1). More precisely, the UF was positioned after 3 and 8 tanks ( $80 \text{ m}^3$  each) containing 18 000 and 13 000 fish weighing from 100 g to 300 g and from 700 g to 1 200 g, respectively.

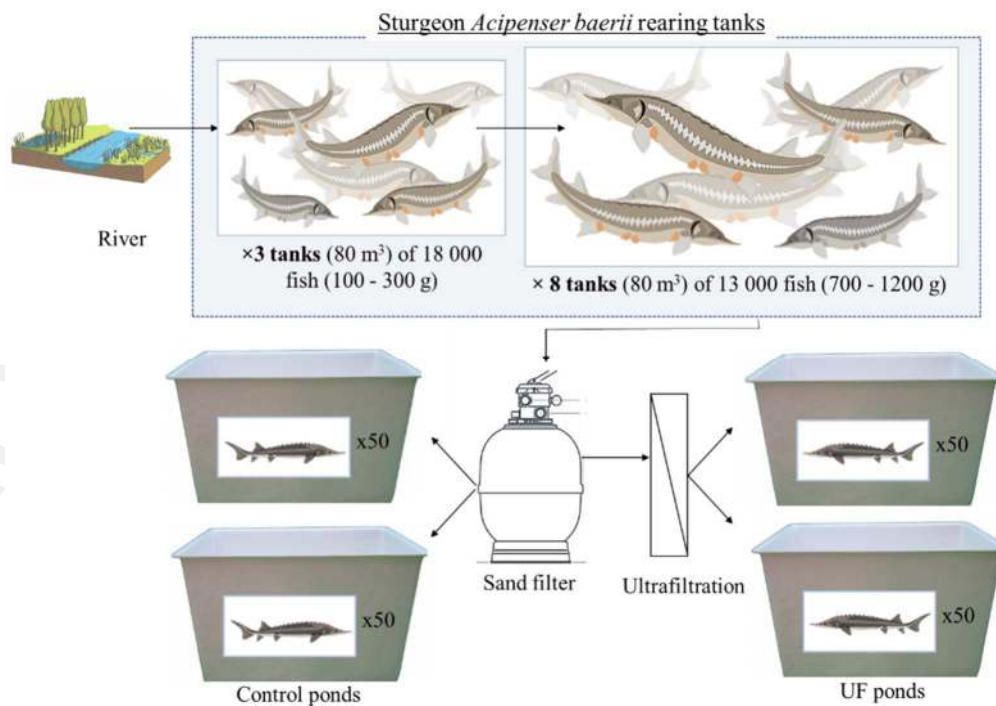


Figure 1: Process configuration of the study

### 2.3. Analysis of fish in control and UF ponds

The ultrafiltered water was sent to two juvenile ponds in parallel. Two other control ponds in parallel were fed with the same sand-prefiltered water only. Each of the 4 ponds (1.1 m<sup>3</sup>, aerated by bubbling, water flow rate around 240 L h<sup>-1</sup> i.e. a water residence time of about 4.6 hours) initially contained 50 Siberian juveniles (*Acipenser baerii*) of 30 g each. There is no significant difference in body mass between ponds or treatment at the beginning of experiment. The 250 fish arrived from the Hennequart fish farm (Sologne, France July, 13<sup>th</sup>, 2021). 50 fish were analysed for initial sampling for qPCR analysis of Mimivirus *Acipenser iridovirus*-European (AcIV-E) and mycobacteria *Mycobacterium liflandii*. The qPCR analysis were performed by an external laboratory (Laboratoire départemental des Landes, Mont de Marsan, France) on pooled organs: gills, liver and intestine. The DNA extraction and qPCR technique are described by Bigarré *et al.* (2017) for AcIV-E. 200 other fish were distributed among the 4 experimental ponds. The average weight is 32.6 g with a standard deviation of 4.2. The pathogenic microorganisms' retention performance of the pilot was evaluated by sanitary monitoring with (i) weekly water sampling before and after treatment for total aerobic mesophilic flora analysis by a certified laboratory (Laboratoire Départemental d'Analyse et de Recherche, Coulounieix-Chamiers, France – standard *ISO 4833-1*) (ii) fish sampling for qPCR analysis of Mimivirus (AcIV-E) and mycobacteria: one fish per pond was analysed at the end of August 2021 by PCR. The analysis of a single fish is to get an idea of the presence or absence of pathogens and not to obtain quantitative information. A new qPCR analysis on a pool of 5 fish per pond was sent to the laboratory at the end of September 2021. Finally, 30 fish per pond divided into 6 pools of 5 fish were sent for analysis at the end of October 2021. Except for the month of August when only one fish was analyzed, a minimum of 5 fishes were analyzed to obtain a rigorous statistical study. 50 and 30 fish were analyzed respectively initially and in each pond at the end of the study in order to obtain reliable results. The qPCR result is given with Ct (cycle threshold) values which corresponds to the number of multiplication cycles necessary to detect the virus in the sample. The Ct value of a reaction is defined as the number of cycles at which the fluorescence of a PCR product can be detected above the background signal. The Ct or threshold value is inversely proportional to the amount of DNA present in a sample, meaning that if the amount of virus is abundant, fewer cycles are required for amplification and quantification, resulting in a lower Ct value. If the sample contains less virus, the Ct value increases, as more cycles are needed to advance the reaction. 3 Ct of difference between two samples is equal to 1 log of concentration difference or 90% removal rate. The detection limit of qPCR analysis is a Ct of 38. On arrival of the fish, no mycobacteria or mimivirus were found in the fish tested and moreover, no external or internal signs or lesions are presented. Weekly monitoring of growth and Feed Conversion Ratio (FCR), corresponding to the weight of the input divided by the output (thus weight of feed per weight of sturgeon biomass) (Dvergedal *et al.*, 2022), was carried out and the daily behaviour of the fish was also observed. For example, a FCR of 0.8 means that 0.8 kg of feed produced 1 kg of sturgeon biomass. At the opposite, a FCR of 2 indicates that 2 kg of feed produced only 1 kg of sturgeon biomass. During the first months of growth, when temperatures are favorable, the FCR is often less than 1. The high biomass uptake is attributed not only to food intake, but also to fish hydration. Fish were fed to satiation based on the FCR. For each of the 4 ponds (2 control ponds and 2 UF ponds), 5 fishes are weighed once a week in a random way and that is repeated on 4 weighings (20 fishes – around 50% of each pond) in order to have a statistical study and reliable data. The daily behavior of the fish was also observed: an appetite indicator ranging from 0 to 3 was constructed. An index of 0 corresponds to a lack of palatability,

with the fish losing interest in the food and not feeding. On the contrary, an index of 3 corresponds to a high appetite and good food intake. The total duration of the study is 5 months from July 2021 to November 2021.

### **3. Results and discussion**

#### **3.1. Fish growth**

Growth is an important factor in fish farming. One of the main issues is to increase growth rapidly and reduce the age of maturity in order to keep the fish as short as possible in the farm and thus reduce the production cost of a batch. In these trials, as described in the previous section, around 50% of each pond was weighed weekly to establish an average weight and average growth. The variation of the growth (average values of the two UF ponds and average values of the two control ponds) as the function of time is given in Figure 2. The Feed Conversion Ratio (FCR) was calculated to be identical from one pond to another with a ratio of 2.2 in the hot period ( $>21^{\circ}\text{C}$ ) then decreasing with the drop in temperature to reach 1.2 in the cold period at the end of October ( $<13.5^{\circ}\text{C}$ ). The fish feed is mainly added based on this FCR. However, mass monitoring is carried out to evaluate if ultrafiltration does not remove naturally occurring food from the river that would limit growth. Growth is slightly better for the ultrafiltered water (Figure 2 – UF ponds) compared to the control water (Figure 2 – Control ponds). This improvement is validated by a two way ANOVA statistical test performed with R software (Table 1). The ANOVA test shows that the influence of the “day” variable on the average weight of the fish is significant as the value of F test is much higher than 1 and the p-value is much lower than 0.05). Indeed, the more the study duration is, the more the average weight increases. The influence of the “water” variable on the average weight of the fish is also significant (Table 1). Thus, the better growth in ultrafiltered water than in the control tank observed on the growth curves is statistically confirmed. Although not shown, the standard deviation was relatively large for all ponds with fish ranging from 150 g to 450 g at respectively at the beginning and at the end of the study. Together, the growth rate and feed conversion rate suggest that UF water is suitable and this conclusion is in agreement with a previous study carried out on oyster production in which the quality of ultrafiltered water was found to be suitable for different aquaculture applications (Cordier *et al.*, 2019b). Although sturgeons and oysters are different species, bivalves are also sensitive to degradations in water quality, and oyster hatchery/nursery production depends on seawater of consistent quality, free from parasites and pathogens. Ultrafiltration, in addition to proving its performance in eliminating viruses and bacteria problematic for this application, has demonstrated that the water produced can be used for spat growth, fertilization or microalgae cultivation.

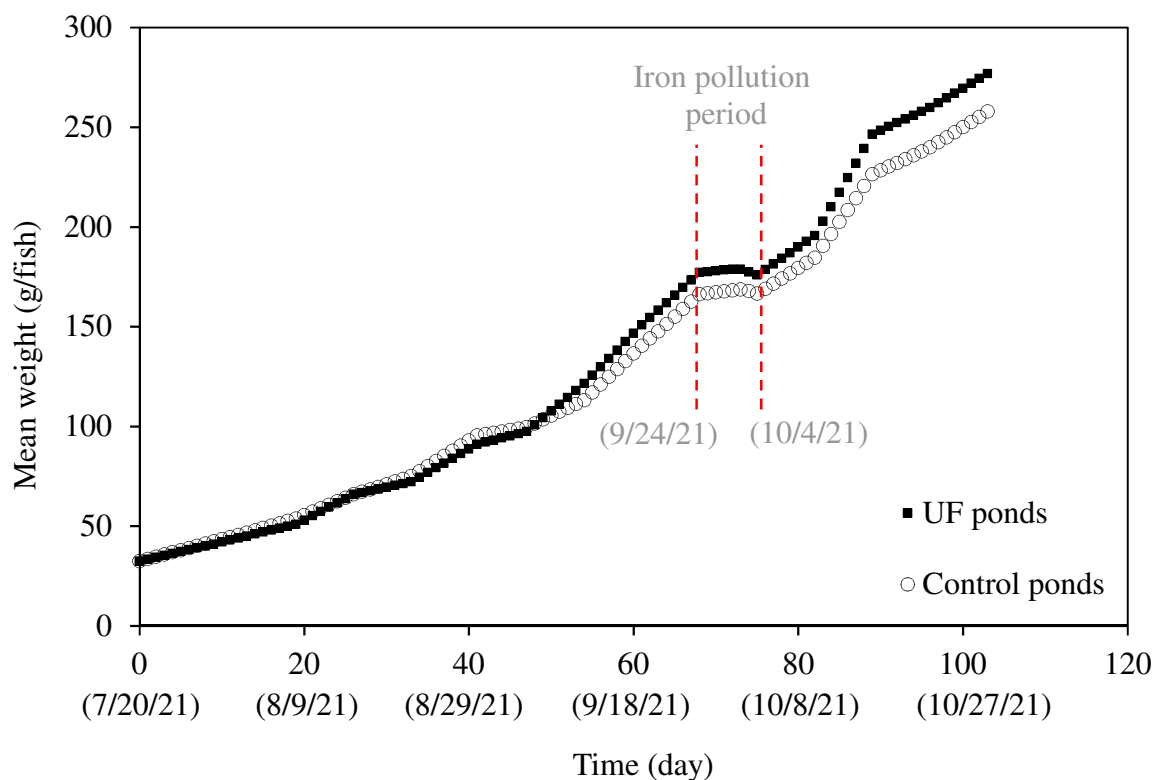


Figure 2: Variation of the fish weight for UF and control ponds as the function of the date

Table 1: Statistical value for the influence of “day” and “water quality” on the fish growth

	Value of F test	p-value
Average weight as a function of the day	10839	$< 2.2 \times 10^{-16}$
Average weight as a function of the water quality	18	$< 2.5 \times 10^{-5}$

This weight parameter is very sensitive as it can be seen for example at the end of September (at day 70) when an accidental iron pollution of the river caused a significant mortality of the fish in the farm and therefore a stop of the feeding for a few days (Figure 2). This is clearly visible on the growth curve which stops at the moment of the pollution. Only total iron was analyzed in the raw water, at a concentration of around  $0.5 \text{ mg L}^{-1}$ . It is highly likely, however, that most of the iron was in the form of iron complexed with organic matter, as the UF membrane was heavily fouled (see section 3.4), and in the form of ferric ions passing through the membrane, as histological examinations of the gills of affected fish revealed ferric clusters on the gill epithelium.

Finally, the comparison of the fish behaviour in the different batches was evaluated and especially the palatability of the fish. The feed palatability was assessed visually and classified into four categories: (3) very good: strong interest for the feed, consumption as soon as it is distributed, no pellets remaining at the end of the day, (2) good: high interest for the feed, degrading progressively during the day, no pellets left at the end of the day, (1) bad: low interest for the feed degrading during the day, a part of the ration is not consumed, (0) very bad: no interest for the food, the ration is not consumed. Throughout the study the fish showed a very good feed palatability for the feed in all ponds and a value of (3) was obtained throughout the whole study in all ponds even with iron

pollution. However, food intake leads to a higher demand for oxygen. Iron clogging of the gills reduces the fish's access to oxygen.

### 3.2. Total flora retention

During this study, the total aerobic mesophilic flora concentration ranged from 5 200 to 170 000 000 Colony Forming Unit (CFU) 100mL<sup>-1</sup> after the sand filter. It should be noted, however, that the inlet concentration is very high due to the passage through the eleven sturgeon *Acipenser baerii* rearing tanks. Obviously, the highest concentrations were observed between August, 18<sup>th</sup> and September, 15<sup>th</sup> when the water temperature was the highest at around 21°C. As shown in Figure 3, the logarithm removal rate varies from 2 to 4.7, the retention of total flora by UF is high but not complete. The dash line aims to give a trend. Empty squares indicate underestimated logarithm removal values (LRV) values due to concentrations in the permeate below the detection limits of the analysis. The significant bacterial reduction at the UF membrane outlet provides a bacterial control and highly simplifies the supply of probiotics. The use of probiotics to control the fish's internal bacterial flora is becoming increasingly common in fish farming. In this way, the installation of a selected bacterial population favorable to the development of fish is favored when the environment is low in bacteriological load. This is the case after UF.

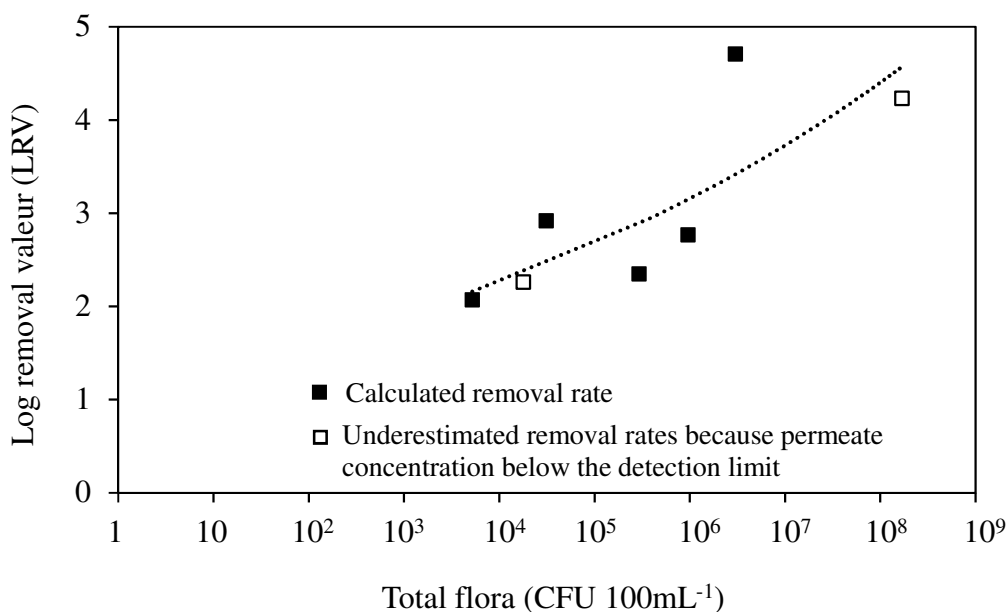


Figure 3: Logarithm removal rate of total flora by ultrafiltration as the function of the inlet total flora concentration

In the control ponds supplied with water not treated by UF, green algae appeared on the entire edge of both ponds. In contrast, these macroalgae were absent in the ponds supplied with ultrafiltered water. The dissolved oxygen concentrations were highly variable throughout the day in the control ponds compared with the UF ponds as shown in Figure 4. They can be explained by temperature variations during the day and the presence of organic matter, but these variations are greater in the control ponds and could be related to the presence of this algae and river phytoplankton communities retained by the ultrafiltration process.



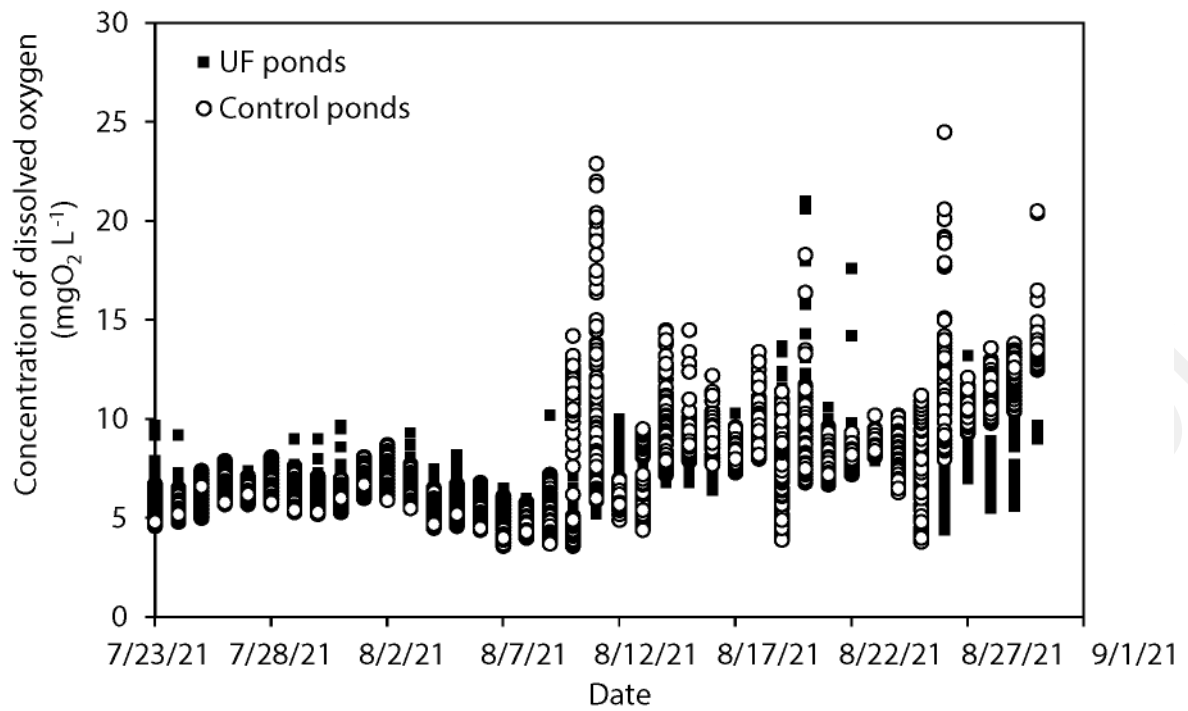


Figure 4: Variation of the dissolved oxygen concentration in UF and control ponds as the function of the date

### 3.3. Mimivirus and mycobacteria retention

As with the mycobacteria, no external/internal signs suggestive of mimivirus were detected upon arrival of the fish. However, individual qPCR analyses showed that 5 out of 50 fish were carriers of the virus. The amount of virus was however relatively low with a Ct number between 36.1 and 37.9 (average 37). This initial relative absence of virus is confirmed by the results of August and September when no virus detection was observed in the tanks supplied with UF water. Ct number of less than 25 is indicative of a high viral load. In contrast, a Ct number greater than 30 indicates a low viral load. As reminder the qPCR detection limit is a Ct value of 38. After one month of study, the August sampling clearly shows (Figure 5) the presence of mimivirus in the fish coming from the control ponds whereas it is absent in the fish fed with ultrafiltered water. These results are validated by the detailed analysis of the October sampling realized on 120 fish (30 for each pond) presented in Figure 6. Regarding the protection of the farms, the qPCR analyses of 6 pools of 5 fish in the 4 ponds are also shown in Figure 5, which shows the number of multiplication cycles necessary to detect the virus by pool. Fish in control ponds had Ct values between 24 and 29 with an average of 27. Pond 1 fed with ultrafiltered water (UF1) had lower loads, ranging from 31 to 37 with an average of 33, i.e. a decrease of between 1 and 4 log (average 2.5 log) in the presence of viruses. Pond 2, placed in parallel and supplied with UF water (UF2) does not seem to be affected by the virus (average value higher than the detection limit): a decrease of between 3 and 4.6 log (average 3.7 log) in the presence of viruses. Ultrafiltration has eliminated the mimivirus and therefore protects sturgeon farms. With regard to the mycobacterium, the water temperature during the summer of 2021 was not high enough for the mycobacterium to develop. No mycobacterium is detected during the study.

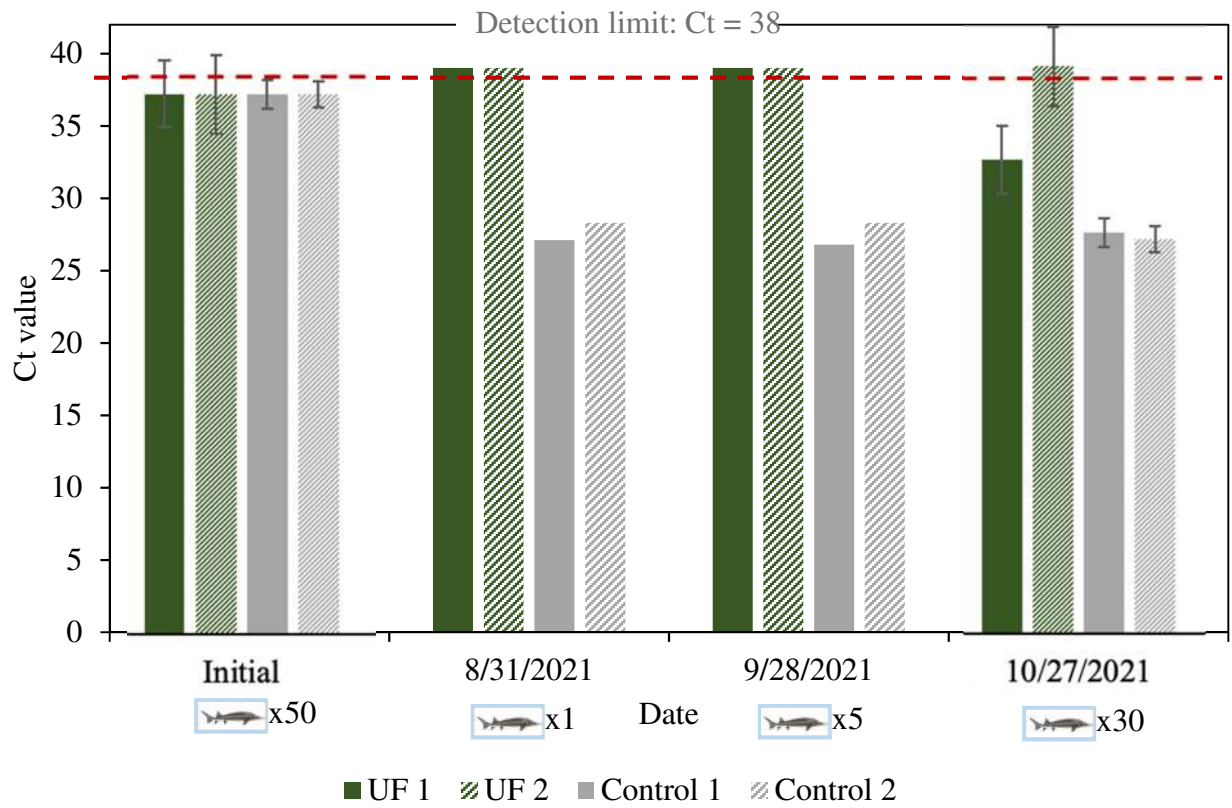


Figure 5: Number of multiplication cycles necessary to detect the mimivirus as the function of time

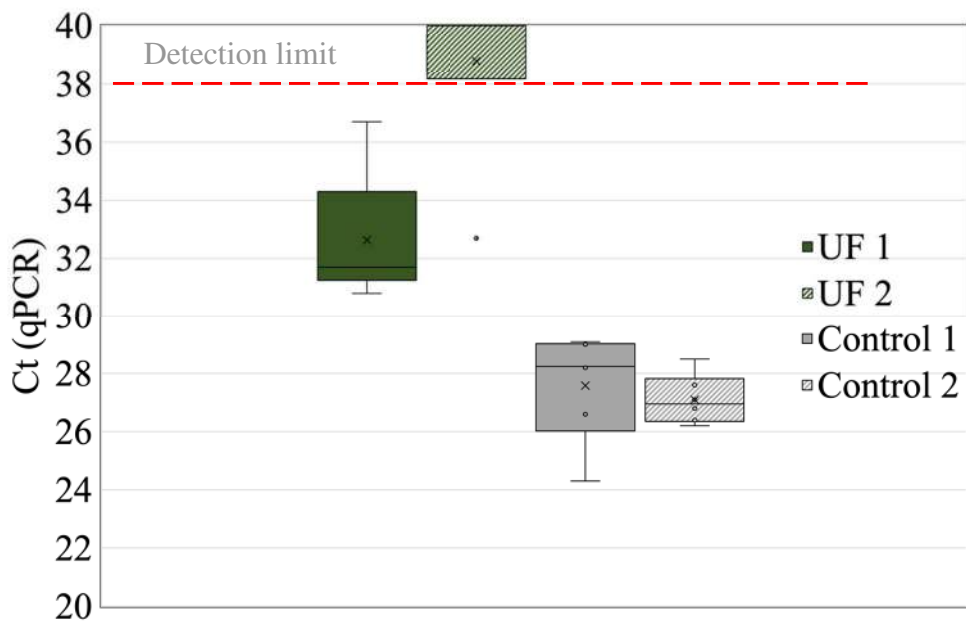


Figure 6: Detailed results of Ct values for the final analysis of October on 30 fish

### 3.4. Ultrafiltration pilot plant hydraulic performances

The UF pilot plant used in this work was studied in terms of hydraulic performance, monitored with different feed water qualities. In terms of hydraulic performance, the process has proven to be resilient in the face of variable feed quality. Figure 7 shows the variation of membrane permeability ( $L_p$ ) and turbidity as a function of time between July and November 2021. Figure 7-a shows a permeability that varies between 400 and 600  $L h^{-1} m^{-2} bar^{-1}$  and always higher than 200  $L h^{-1} m^{-2} bar^{-1}$  which is the limit to trigger a chemical cleaning. Chemical cleanings were nevertheless automatically triggered every 4 days (shown by the arrows – Figure 7-a and Figure 7-b). In August (Figure 7-b), the total flora strongly increases from 5 200 to 170 000 000 CFU  $mL^{-1}$ , resulting in a greater variation in permeability but still in the same range and without reaching the CEB limit. The UF process performs well with these operating conditions (filtration flux,  $J = 60 L h^{-1} m^{-2}$ , filtration time,  $t_f = 60$  min). On September 24<sup>th</sup>, an accidental iron discharge into the river had a severe impact on the ultrafiltration process as shown in Figure 7-c. The permeability dropped very quickly and an average of 4 chemical cleanings per day were required which is twice as important as the sustainable conditions. The permeability varied every 4 hours between from 500-600 to 200  $L h^{-1} m^{-2} bar^{-1}$ . This incident resulted in a very high fish mortality in the farm of up to 400 per day (with a presence of iron found in fish tissues and organs). The UF data immediately pointed to accidental pollution and could thus serve as an alarm on a fish farm. The water analyses showed (September 29<sup>th</sup>) a residual total iron concentration of around 0.5  $mg L^{-1}$ . UF monitoring corroborates the hypothesis that the iron contamination was partly in complexed form with the organic matter, as the very rapid fouling of the membranes during pollution would confirm the retention of these complexes. The operating data for the UF process was immediately affected and could therefore be used as an alarm on a fish farm in the event of this type of accident, thus protecting the fish. Thus, in the event of a sudden and significant drop in the filtration performance of the ultrafiltration pilot, reflecting a deterioration in water quality, the protection of fish could be ensured by imposing closed-circuit operation and avoiding the introduction of contamination. The iron pollution could not be eliminated and it was necessary to wait until the end of the episode at the beginning of October and a return to higher permeabilities. Then, in October (Figure 7-d) a return to the same UF performances as in July was observed, thus demonstrating the flexibility of the UF pilot plant over the duration of the study, but also in the face of accidents modifying the quality of water. Over the duration of the study and whatever the quality of the incoming water (apart from accidents) the permeability varies between 400 and 600  $L h^{-1} m^{-2} bar^{-1}$  in the sustainable conditions used and only one chemical cleaning is automatically carried out every 4 days to avoid long-term irreversible fouling which is a viable operating condition.

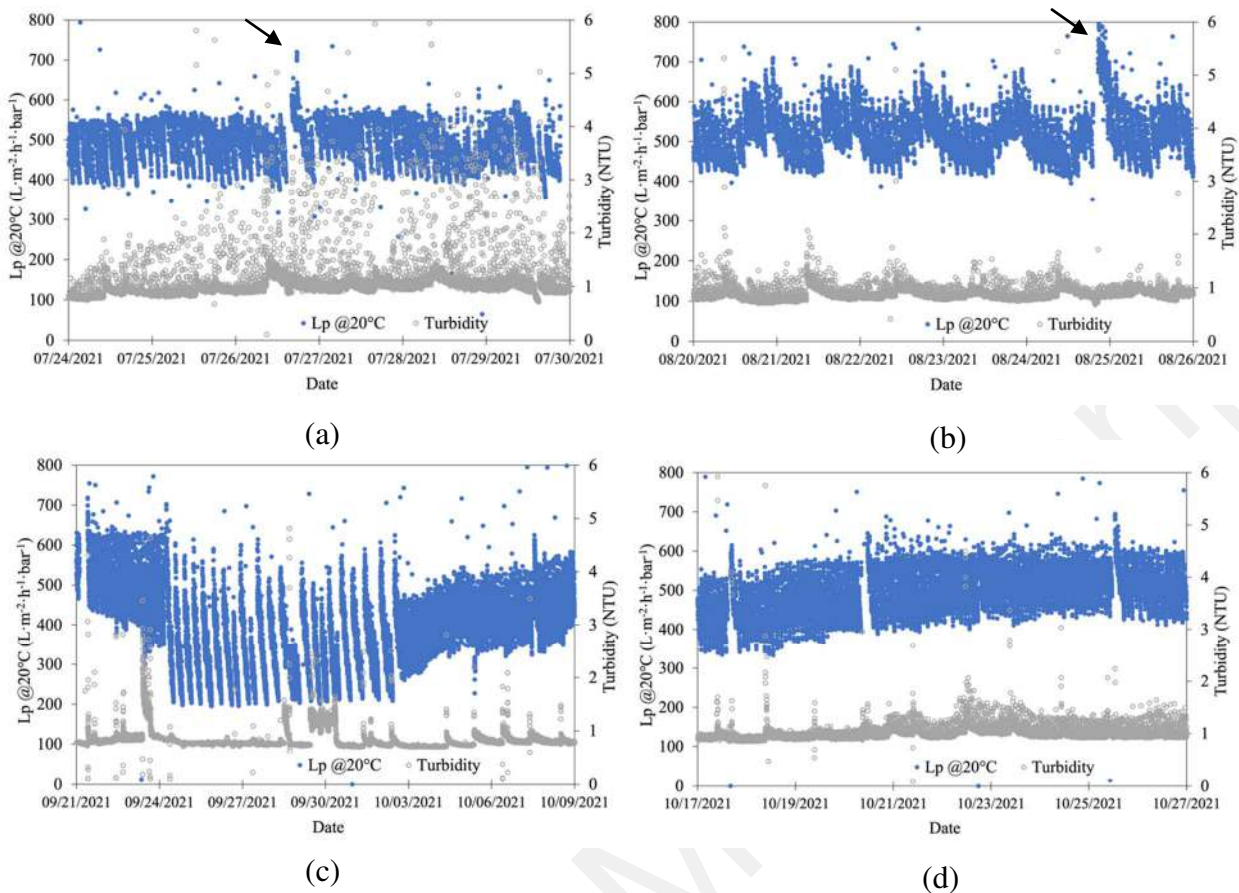


Figure 7: Variation of the membrane permeability and turbidity as a function of time for different months of study [(a) standard conditions, (b) high concentration of total flora, (c) accidental iron discharge, (d) standard conditions]

#### 4. Conclusion

Ultrafiltration provides water quality suitable for the growth of juvenile sturgeon. UF water quality leads to a higher fish growth than water treated only with sand filtration. This difference is statistically validated. The retention of total aerobic mesophilic flora is function of the feed concentration and is between 2 and 4.7 log retention. It should also be noted that algae growth in ponds fed with UF water is much lower, resulting in smaller variations in dissolved oxygen content, and is explained by the retention of phytoplankton populations present in the river water by the ultrafiltration process. In general, in the control ponds, the mimivirus appears very quickly at very high concentrations and remains present throughout the study, whereas in the ultrafiltered water it only appears after 5 months and at very low concentrations, close to the detection limit of the analyses. This corresponds to a continuous abatement of about 4 log throughout the study. During the study, the water temperature did not exceed 21°C and therefore the mycobacterium was not detected. Given the larger size of the mycobacterium (length 2-5  $\mu\text{m}$ ; width 0.2-0.3  $\mu\text{m}$ ) and the mimivirus (AcIV-E) (0.2-0.3  $\mu\text{m}$ ) compared to the UF pore size (20 nm), it can be assumed that the steric retention of the mycobacterium and mimivirus would have been at least identical but still

need to be confirmed. About its resistance, the process has demonstrated its viability and efficiency over 5 months of continuous filtration. Both in terms of the quality of the water produced (elimination of pathogens, control of bacterial flora and a quality suited to aquaculture production), and in terms of the robustness of the process (resistance to variable water quality and long-term economic viability), the results of this and previous studies focusing on oyster production support the idea of integrating the UF process into aquaculture production. This would be of particular interest in the case of recirculation systems, where the process could be used both as a treatment for discharge water and as a treatment for farm renewal water, in order to guarantee the biosafety of fish production with reliable, pathogen-free water quality. A possible outcome of this study could be detailed monitoring of the quality of the water produced, with more comprehensive analyses of the type and concentration of organic matter or phytoplankton, in order to assess the retention performance of UF.

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