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Ultrafiltration to secure shellfish industrial activities: culture of microalgae and oyster fertilization

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Abstract :

Shellfish farming, a key sector of French aquaculture activity, allows the production of oyster spat in a controlled environment. Their production in commercial hatcheries requires control over the quality of the seawater used to sustain crossbreeding, breeding, and the production of fodder microalgae. Therefore, improving the filtration conditions of incoming water is crucial in ensuring the sustainability of production. An ultrafiltration pilot plant was therefore installed at Vendée Naissain. This ultrafiltration pilot plant allows filtration at 0.02 µm; it is used upstream of hatcheries to eliminate pathogens and parasites that can influence the development of cultivated species and downstream to remove oyster gametes in hatchery effluents. The objectives of this work were: (i) to use ultrafiltered seawater for the culture of the microalga Isochrysis lutea (T-Iso) to determine whether better growth than that observed with borehole water, historically used by the producer, can be achieved; and (ii) to determine whether the use of ultrafiltered water results in better fertilization rates of the cupped oyster Crassostrea gigas compared to filtered and UV-treated seawater. Ultrafiltered water has shown definite efficiency for culturing T-Iso with rapid growth and significant reduction in contamination compared to cultivation in well water. The contribution of ultrafiltered water in hatching is more nuanced; ultrafiltered water does not stabilize hatch rates, and its quality is highly dependent on the quality of the seawater used.

Keywords : Membrane process, hatchery, shellfish culture, Isochrysis lutea, Crassostrea gigas

1. Introduction

Population growth and the degradation of existing resources have encouraged humanity to increase aquaculture activities in response to consumption demand. According to the Food and Agriculture Organization (FAO), worldwide aquaculture production reached a new record of 114.5 million tons in live weight in 2018, with a total sale value of USD 263.6 billion. Total production comprises the following: 82.1 million tons of aquatic animals (USD 250.1 billion), 32.4 million tons of aquatic algae (USD 13.3 billion), and 26,000 tons of ornamental seashells and pearls (USD 179,000) [1]. Aquaculture activity is an important socioeconomic and cultural activity in the coastal zone; this activity includes farming oysters, which are considered a luxury product [2]. However, shellfish production is strongly linked to the environment and is sensitive to changes in the marine environment. Oyster farming is indeed affected by mortalities, sometimes resulting in the extinction of species such as *Ostrea edulis* and *Crassostrea angulata*, respectively, in 1920 and 1971 in France [2–4]. Oyster producers face various risks and limiting factors, such as infectious diseases [3]. Since the 1970s, the oyster *Crassostrea gigas* has been introduced to avoid a decline in production. Moreover, hatcheries and nurseries can produce diploid and triploid oysters because of their higher resistance to diseases to complete or replace wild-caught spat on oyster farms. According to the FAO 2019 report, *C. gigas* represents 98% of the French oyster production. Unfortunately, this species is affected by pathogenic organisms such as the Ostreid herpesvirus 1 (OsHV-1), which has generated mortality rates of over 70% in spat and juveniles, and the bacteria *Vibrio aestuarianus*, which has led to similar mortalities of adult oysters since 2012 [5,6]. These two pathogenic agents have a significant effect on oyster production, which decreased from 111,000 tons in 2007 to 64,000 tons in 2016 [6,7]. More information on viruses and bacterial families has been reported in several papers [8–11]. The removal of pathogenic species is highly recommended in the first stage of life of larval animals because of their vulnerability at this stage [12,13]

On the other hand, in aquaculture, microalgae are cultivated and are the foundation of the diet of many species, particularly bivalve animals. The most cultivated microalgae are *Skeletonema*, *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Phaeodactylum*, *Monochrisis*, *Pavlova*, and *Isochrysis*, which are targeted for their nutritional quality [14–16]. The culture of microalgae can be performed according to batch, semicontinuous, or continuous cultures in photobioreactors [17–20]. This method is made possible by controlling parameters such as culture time, the level of dissolved carbon dioxide, or even the quantities of inoculum introduced when transplanting the cultures. This growth control ensures the daily availability

culture to feed various aquaculture species. The challenge in microalgae culture is to maintain the reliability of culture for a long duration, regardless of the change in water quality [15,21]. Considering this context, water quality seems to play a very important role in aquaculture activities. Nowadays, the degradation of water quality is increasingly observed and can directly affect the culture of microalgae and larval rearing [22–24].

The management of the water quality is an important issue that contributes to improving biosecurity within an aquaculture facility. Biosecurity encompasses all measures aimed at preventing the appearance of diseases and those aimed at limiting their spread by isolating farm animals as much as possible. Water treatment is therefore an essential element in the chain of management measure, particularly during sanitary crisis where protection of production systems (animals and microalgae) from pathogenic microorganisms and parasites that may come from seawater or others activities on the farm, is essential [25].

The common treatment used in shellfish hatcheries and nurseries is filtration to remove larger particles, followed by UV disinfection to inactivate biological microorganisms. The filtration process can use a sand filter, followed by a filtration cartridge to retain particle sizes between 10 and 1 µm [23,24,26]. However, UV radiation is not able to remove viruses and bacteria and only inactivates them. In addition, the efficiency of filtration depends on the quality of the filtration steps carried out before, because the presence of particles can affect the intensity of UV radiation [27]. Moreover, UV radiation can generate toxic molecules [28]. To avoid this limitation, Cordier *et al.* investigated the benefits of using ultrafiltration (UF) in aquaculture activities, which can provide a good and stable quality of seawater [29–32]. Ultrafiltration is a membrane process which a molecular cut off around 10 nm. UF can effectively remove pathogenic microorganisms and pathogenic viruses such as Giardia and bacteria in water [33]. UF has been widely used in pharmaceutical applications, biotechnology, and sugar refining [34] and in the fields of water treatment, such as drinking water production [35,36] seawater desalination [37–40], and water reuse [41]. In our knowledge, UF pilot plant was used by Cordier et al for the first time in hatchery for oyster production. The results show the role of ultrafiltration to protect the marine biodiversity by removing plyploïd oyster gametes in hatchery effluents. The UF process is able to remove about 5 logs gametes with a moderate impact on membrane fouling[25]. However, the challenges on UF process are the fouling management and the investment cost.

In this context, the purpose of the present study was to investigate the performance and impact of the UF process on the biosecurity of aquaculture activities (microalgae cultivation

and hatching of *C. gigas*) at an industrial scale by using a pilot plant with a capacity of 20 $m^3.d^{-1}$ and to compare the results with the classical seawater treatment process.

2. Material and method

All these experiments were conducted at the Vendée Naissain (VN, Bouin, France) hatchery.

2.1. Water type

The water supplies presented here are the ones commonly used at VN for shellfish farming. The water qualities differ depending on the industrial activities targeted: borehole seawater for microalgae cultivation and seawater for oyster production. In this study, we compared two different seawaters used in industrial production with ultrafiltrated seawater. **Borehole seawater (BW)**: The water used for the cultivation of *Isochrysis lutea* (T-Iso) comes from groundwater. This type of borehole provides high-quality water with stable temperatures and salinities. The water from the borehole is naturally filtered through sediment and porous rocks, and this groundwater is therefore used without additional filtration for the culture of microalgae. In the case of small volumes of microalgae cultivation (250 mL and 2 L), BW was boiled. In the case of 30 L, no sterilization was performed.

Seawater (SW): The SW used comes from a channel 2 km in length from the sea (Atlantic Ocean, Bourgneuf Bay, France) and was decanted for 24 h in a pond before use. SW from this pond was used to supply the semi-industrial UF pilot plant, as well as for fertilization. In the latter case, SW was filtered through a sand filter, filtered through 10 µm and 1 µm filters, and treated with UV rays. The experiments were carried from June to end august 2020. This period was known by punctual formation of storm in the region.

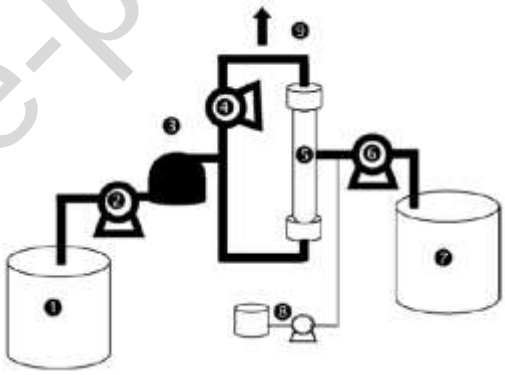
Ultrafiltered seawater will be referred to as **UFSW**.

2.2. UF: membrane and pilot plant

The UF pilot plant used in this study has a membrane module of polyether sulfone (PES) in the form of hollow fibers. Water is filtered from the inside of the fiber to the outside, passing through 0.02 µm pores. The UF module has more than 300 fibers, representing an active filtration area of 8 m². The pilot plant is fully automated (working 24h/24h) (Figure 1) and automatically performs backwashes (injection of ultrafiltered water in the direction opposite

to filtration in order to remove fouling), air backwashes (backwash with a previous injection of air), and chemical cleaning (CEB, injection of chemicals into the membrane) when the permeability of the membrane decreases. The chemicals used for CEB are sulfuric acid, Chlorine and Soda. furthermore, the pilot plant can supply up to 20 m^3d^{-1} of UFSW. The scheme Figure 1.b) shows the different parts of the pilot dedicated to these cleaning steps (backwashing pump, tank and chemical cleaning part). More details can be found in the previous study [29]. The membrane retains biological contamination in particular viruses and bacteria that can influence the development of the cultivated species T-Iso and *C. gigas*. The UF pilot automatically records several data, such as permeability and transmembrane pressure.

(a)



(b)

Figure 1: (a) Picture and (b) scheme of the UF pilot plant. {1—feed tank; 2—feed pump; 3—pre-filter; 4—recirculating pump; 5—membrane module; 6—backwashing pump; 7—purified water tank for backwash; 8—chemical cleaning part; 9—purge}.

2.3. T-Iso cultures

T-Iso was cultured in batch culture at the VN hatchery. Each culture inoculum from the lower volume was transferred to a higher volume (from 250 mL to 30 L) over a 4-day cycle (Figure 1). Four (A-B-C-D) cultures were obtained in duplicate to consider the reproducibility of measurements.

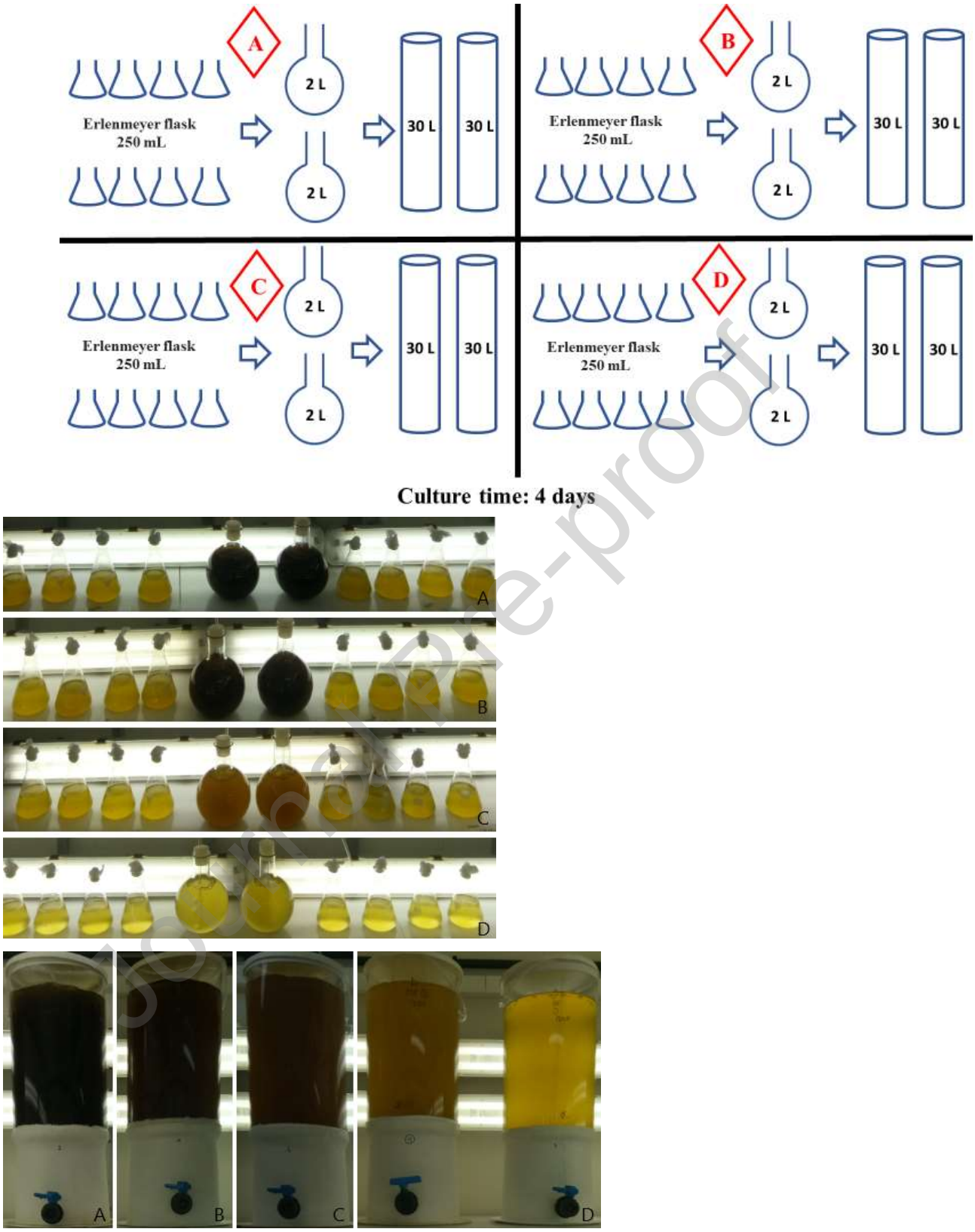


Figure 1: Organizational diagram of subcultures on D4. Four cultures in an Erlenmeyer flask on D4 allow inoculation of four new Erlenmeyer flasks and a 2 L flask at the same time. Once at D4, the balloon will inoculate a 30 L cylinder.

To avoid any problems linked to the origin of cultivated species, a strain of T-Iso was recovered from Ifremer (Bouin, France). This strain, previously cultivated in seawater in the Ifremer laboratory, was subcultured in UFSW from VN and in BW to acclimatize it to different culture media. This acclimatization was carried out using two Ifremer Erlenmeyer flasks (250 mL) at 7 days old for one week. In each container, a culture medium suitable for the water used was added, and culture in USFW therefore received Conway (nutrient medium) prepared by Ifremer. This culture medium was composed of three solutions: Conway, metals, and vitamins [42]. Cultivation on BW received Provasoli, Conway (composed of a metal solution), and vitamins prepared by VN. This nutrient medium was selected and added to account for the quality of the feed water and obtained after the addition, a similar medium for most of compounds.

Four separate series (A, B, C and D), each containing two replicates, were cultivated one after the other to maximize the use of the experiment room and to get as close as possible to the culture conditions during production. Each series was thus composed of eight Erlenmeyer flasks (250 mL), two flasks (2 L) and two cylinders (30 L) for each of the conditions. Each series was cultivated for 4 days before being transplanted to a larger volume. Growth monitoring was performed on the 2 L and 30 L flasks of each series, but not for the Erlenmeyer flasks, for 4 days by measuring the concentration of cells per mL at different phases of the culture. For each series, a sample was taken from each container (four flasks and four cylinders) and fixed using lugol (10 $\mu L mL^{-1}$). Two sub-samples of this sample were placed on a Malassez cell (1 µL). Three squares from the cell grid were counted randomly and then averaged to obtain the concentration in million cells per milliliter (Mcells mL^{-1}). The concentration is given by the following formula:

$$C = N_{avg} \times V_{cell} \times V_{sample}$$

where N_{avg} is the average number of cells counted per square, and V_{cell}, V_{sample} are the volume of the cell and the sample, respectively. The average number of cells counted by squares (0.01 µL) multiplied by the volume contained in the Malassez cell (1 µL) and by the volume sampled (1 mL). This gives a concentration in Mcells mL^{-1}. The average specific growth rate (μ (s^{-1})) of the cultures was calculated according to the following formula [43]:

$$\mu = \ln (N_t / N_0) / \Delta t$$

where N_0 is the biomass at time t_0, N_t is the biomass at time $t > t_0$, Δt is the time interval $t - t_0$, and μ is expressed in $Time^{-1}$. The instantaneous growth rate (μ) can be converted to the growth rate, expressed as the number of doublings per day (k), using the following formula:

$$k = \mu / \ln(2) = \mu / 0{,}693$$

2.4. Hatching of *C. gigas*

Two types of fertilization were performed: diploid fertilization with gametes from diploid oysters and triploid fertilization using diploid females and tetraploid males. The first step in the fertilization protocol is stripping, which involves the removal of oocytes or sperm from the gonad with a scalpel. The oocytes of five female oysters were collected on a 100 µm or 130 µm sieve (recovering the waste) superimposed on a 15 µm sieve to collect the oocytes. The front side of the female oyster was stripped in UFSW and the back side in SW. Second, three males were stripped on a 500 µm sieve to extract potential waste and collect the sperm in a beaker. Similar to females, one face of the gonad was stripped in UFSW and the other in SW. The female and male gametes were collected in separate and diluted beakers. The oocytes extracted from the gonad had a drop shape, and hydration in the beakers allowed them to return to a round shape and prepare them for fertilization. The oocytes were counted on a Malassez cell, the entire cell was counted, and the number of oocytes was estimated in millions and related to the total number of oocytes from the beaker. Fertilizations were realized by introducing an equivalent number of oocytes from each female in each beaker. For each condition, the oocytes of the five females were then mixed and resized to concentrate them in a minimum amount of water and to introduce the sperm, always in excess. Fertilization lasted approximately 20 min, until the polar bodies were observed. After that, the eggs were again sieved through a 15 µm mesh to remove the superfluous sperm and divided into three beakers for each condition of water quality. The quantity of oocytes in each beaker was estimated by counting on a Malassez cell. After 24 h, the jars were withdrawn through a 44 µm sieve to recover the larvae. D larvae and abnormal larvae were counted on a Sedgewick cell with 3 samples of 20–50 µL depending on the concentration. A minimum count of 100 larvae per sample was considered reliable. The fertilization and hatching rates were estimated using the following equations:

$$T_F = N_L / N_O \times 100$$

where T_F is the total fertilization rate, N_L is the number of hatched larvae, and N_O is the number of oocytes.

$$T_H = N_D / N_O \times 100$$

where T_H is the hatching rate (viable larvae), N_D is the number of viable D larvae, and N_O is the number of oocytes.

The abnormality rate was estimated by the following equation:

$$T_A = N_{DL} / N_L \times 100$$

where T_A is the abnormality rate, N_{DL} is the number of deformed D larvae, and N_L is the total number of larvae counted.

2.5. Analyses

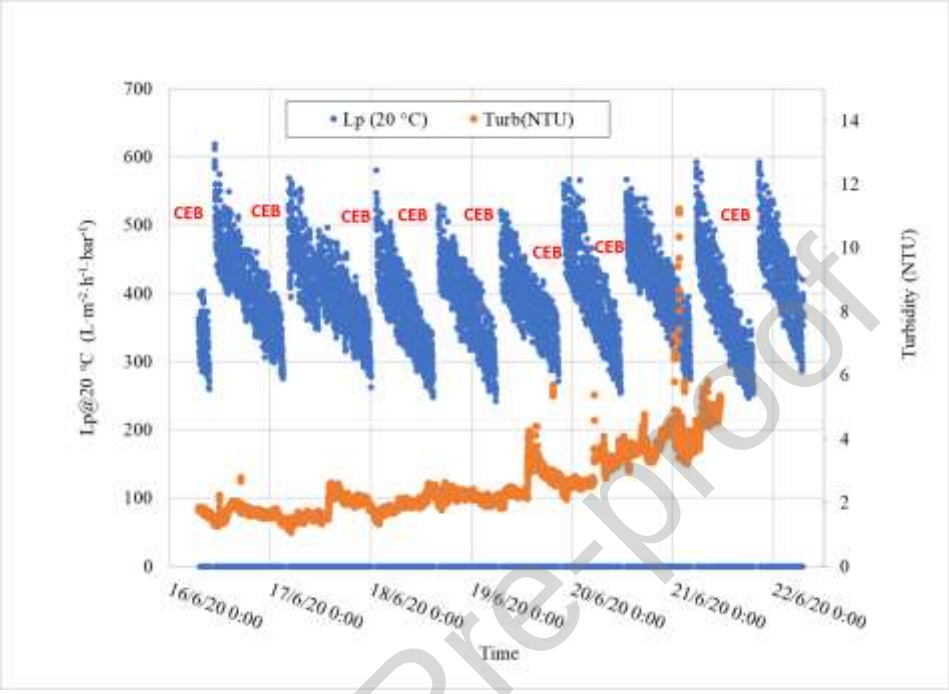
The parameters of BW and UFSW, temperature, pH and salinity were measured using a probe and pH meter. Bacteriological points were determined by counting to visualize bacterial growth in BW and UFSW. The heterotrophic or TCBS agar culture media for total load bacteria and *Vibrio* counting were used, respectively. Petri films with samples placed in pure culture and diluted to 10^{-1} and 10^{-2} were used. The Petri films were placed in an oven at 30 °C for 48 h, and then the number of bacterial colonies was counted or estimated if the whole Petri film count was impossible. The number of colonies was expressed in colony forming units per milliliter (CFU mL^{-1}).

3. Results and Discussion

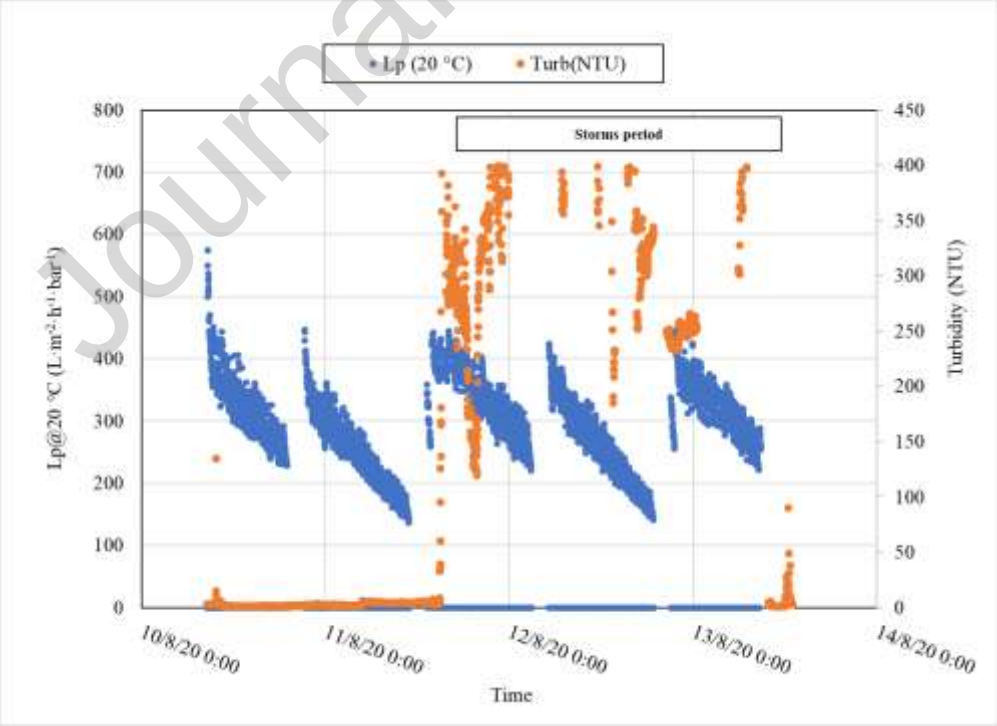
3.1. Performance of UF pilot plant

As indicated above, seawater was obtained from the Atlantic Ocean by a 2 km channel and allowed to decant in a pond for 24 h. The water was then pumped directly into the pilot plant to continuously fill a feed tank, then the seawater was sent through a 130 µm pre-filter and then to the membrane in frontal filtration mode. The flow rate was 60 $Lh^{-1}m^{-2}$ with a filtration duration of 30 min; the backwash is programmed every 30 min and alternated with an air backwash (AB) at a frequency of 1 AB for three backwashes. These parameters were optimized by Cordier *et al.* [32]. The membrane permeability (Lp) was calculated and recorded continuously every minute. All the results are expressed considering the variation in temperature. Permeability was normalized by considering the viscosity fluctuations with temperature. The variation in permeability with time and turbidity (Figure 3) showed that permeability was between 600 and 280 $Lh^{-1}m^{-2}bar^{-1}$, and one CEB (chemical cleaning) is necessary to recover the initial permeability every 24 h. The turbidity was lower than 6 NTU.

However, during the storm period, especially at high temperatures (the recorded temperature of the feed UF pilot plant was 31 °C), the permeability decreased to approximately 200 $Lh^{-1}m^{-2}bar^{-1}$ and the turbidity reached 6 NTU.



(a)



(b)

Figure 3: Evolution of permeability (20 °C) and turbidity versus time (permeate flux = 60 $Lh^{-1}m^{-2}bar^{-1}$ and filtration time = 30 min) for the favorable (a) and storm periods (b).

Total flora and *Vibrio* analyses were performed on different points of the pilot plant: in the feed tank (seawater), after the membrane module, in the permeate tank, and outside of the permeate pipe. The results presented in Table 1 clearly show that the membrane can completely remove *Vibrio* bacteria and allow a high rejection of total flora. UF can provide better bacterial control. It should be noted that the checkpoint of the permeate pipe confirms that the UF pilot plant and pipe should be cleaned periodically to avoid the development of bacteria in the system.

Table 1: *Vibrio* and total flora measurement

	Total flora concentration (CFU mL^{-1})			*Vibrio* flora concentration (CFU mL^{-1})		
	08/18/2020	08/19/2020	08/20/2020	08/18/2020	08/19/2020	08/20/2020
Raw sea water	13,133	20,000	10,867	427	3,007	933
UF water at membrane outlet	13	200	-	0	0	0
UF water permeate tank	2,547	367	133	0	0	0
Permeate pipe	2,453	5,273	2,493	0	0	0

3.2. T-Iso culture

The culture parameters measured every day in the 30 L cylinders before inoculation showed an average temperature of 22.1 °C ± 1 °C for BW and 21.8 °C ± 1.1 °C for UFSW. The average salinity was 34.7 ± 0.4 ppm for BW and 34.1 ± 1.4 ppm for UFSW. Finally, the average pH was 7.7 ± 0.2 for BW and 8.3 ± 0.4 for UFSW. An overall uniformity of the parameters was observed between BW and UFSW despite the greater variability in UFSW. UFSW is seawater from ponds and is therefore subject to climatic variations, while the drilling water is groundwater, which offers greater stability of these parameters. During the experiment, all T-Iso cultures were observed and scored according to four criteria: appearance, mobility, contamination, and effective use (to produce a larger volume or its use as feed) (Figure 4). For the appearance, three scores can be given: (A) good color of the cultures to the naked eye and no deposit at the bottom of the container; (B) color ok, a little deposit at the bottom, and can be effectively used; and (C) ocher to green color, onset of culture crash or cell death, and cannot be used for transplanting or feeding larvae. It was thus observed that the appearance of the cultures was considered satisfactory at more than 90% for all the 2 L flask and 30 L cylinders, as well as for the UFSW Erlenmeyer flask (250 mL). However, only 56% of the BW Erlenmeyer cultures showed a good appearance when this aspect reached almost 100% in UFSW. Mobility decreased as the volume of the culture

increased. A greater mobility of T-Iso cells was observed in the UFSW Erlenmeyer cultures and 65% of the containers exhibited a high mobility, although this mobility decreased for larger volumes (15% in a 2 L flask and 11% in 30 L cylinders of high mobility). Cultures in BW had higher mobility in a 2 L flask than in other containers; 44% of the containers thus have high mobility against 37% in Erlenmeyer flasks and 12% in 30 L cylinders. Contamination, especially that linked to the observation of ciliates in cultures, was 9.2% for UFSW and 13.5% for BW for all containers. However, the most contamination was found in 30 L BW cylinders with 40% contaminated containers, compared to 8% for UFSW. This observation may be explained by the BW treatment; the BW was boiled for sterilization for small volumes (250 mL and 2 L) but there was no sterilization step for 30 L cultivation. Filtration used as a unique treatment without sterilization seems inefficient in eliminating ciliates from seawater and thus do not sufficiently protect the microalgae culture. Whether or not a culture was transplanted appeared to be strongly correlated with contamination. Particular attention was paid to the 30 L cylinders, which are used to inoculate the 300 L cylinders in production or are directly supplied to the breeding oysters. Figure 5 summarizes all the scores assigned to 30 L BW and UFSW; the results show a more regular appearance in UFSW than in BW, with nearly 97% of A. The results also show a greater mobility of the cultures in 30 L on UFSW, with 44% of cultures having high mobility versus 28% in BW. Finally, less contamination of the cylinders was observed in UFSW.

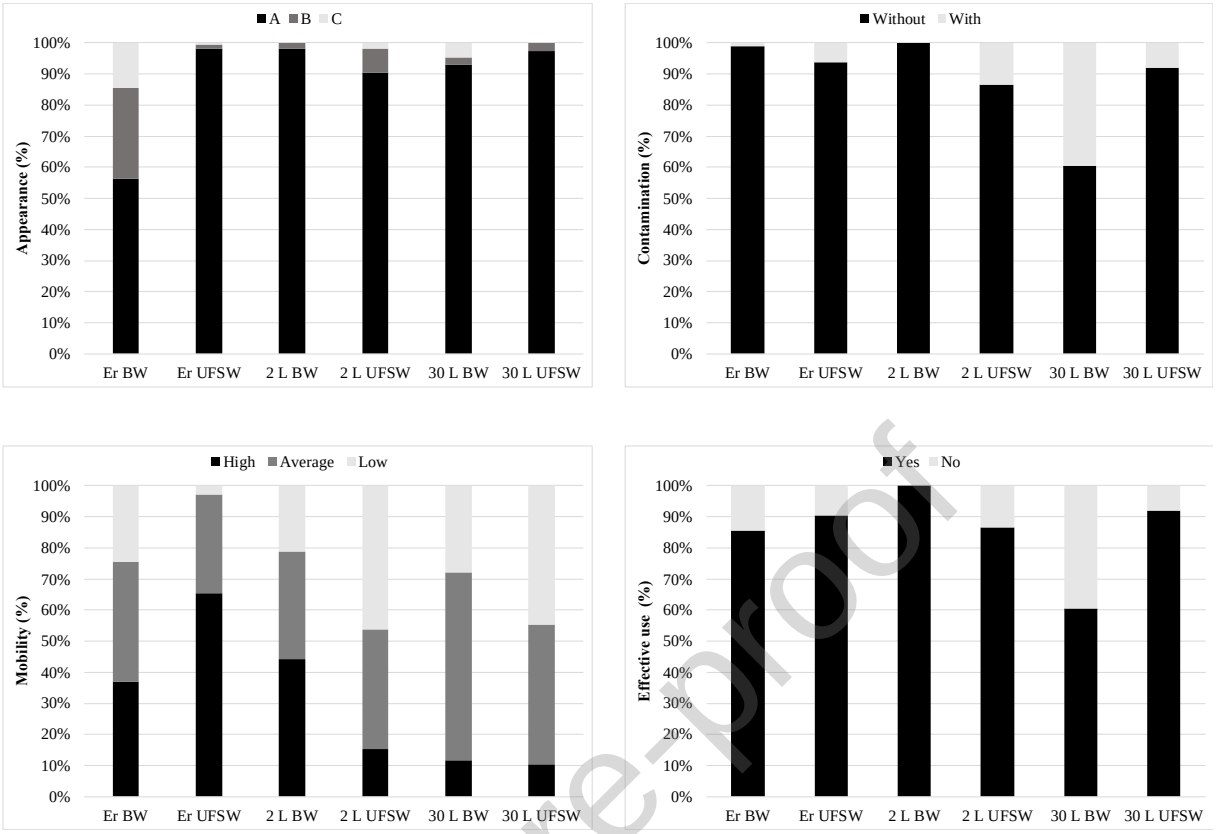


Figure 4: Appearance, mobility, contamination, and effective use of T-Iso cultures in the different containers as a percentage of scores assigned on all replicates.

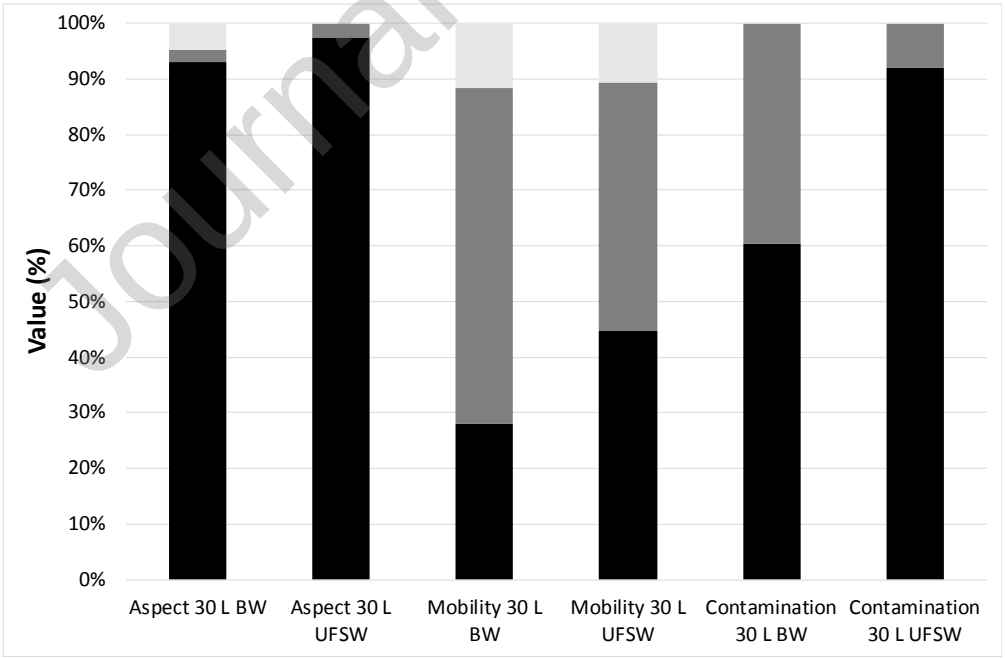
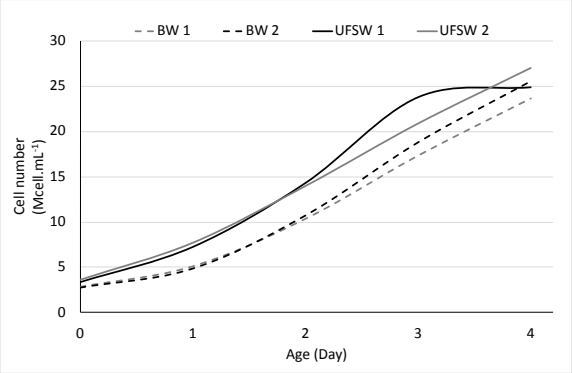
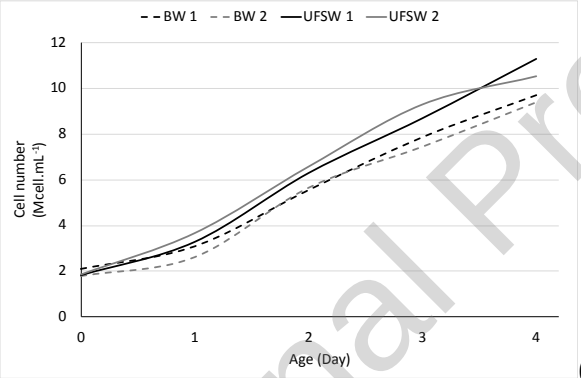


Figure 5: Appearance, mobility, and contamination of the 30 L UFSW and BW. The colors black, gray and white represent the appearance (good aspect A, average aspect B, crash of culture C), mobility (good mobility, medium mobility. low mobility), and contamination (uncontaminated, contaminated), respectively. All results are expressed as a percentage of the containers.

When monitoring the growth of the four series, the data came from 160 samples counted twice. The average standard deviation obtained from these counts was 0.242 Mcells mL^{-1}. The growth curves of each series (Figure 6) show identical kinetics, and the adequacy between the replicates makes it possible to establish an average growth curve for the 2 L flasks and 30 L cylinders.



(a)



(b)

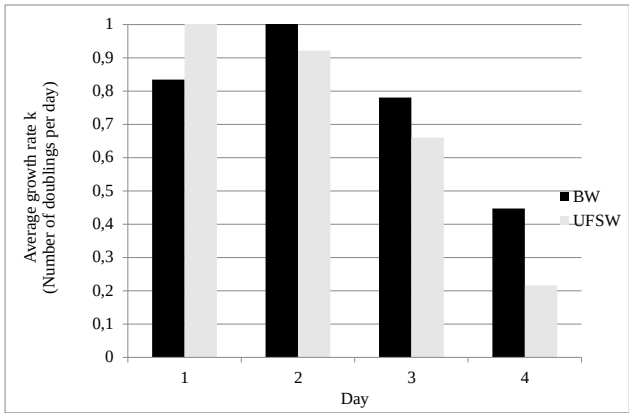
Figure 6: Average growth curve of T-Iso for the two replicates and the two types of water in a 2 L flask (a) and 30 L cylinder (b).

The curves show faster growth of cultures in UFSW with similar day 4 (D4) concentrations between the two conditions in a 2 L flask. The number of cells per milliliter was similar between the two conditions in a 2 L flask until D3; at this point, growth of the UFSW culture slowed down, while the BW culture continued to grow. As the concentration of cultures in a 2 L flask was high (25 Mcells mL^{-1}) and sharply increased until D3, this can influence the arrival of the constant value in a 2 L flask and not in a 30 L cylinder, where the concentration was lower (10-12 Mcells mL^{-1}). This was validated by results obtained with similar concentrations of approximately 21 Mcells mL^{-1} for different seawater purities (Figure 8). For the 30 L cylinders, a higher concentration of cells per milliliter was observed in

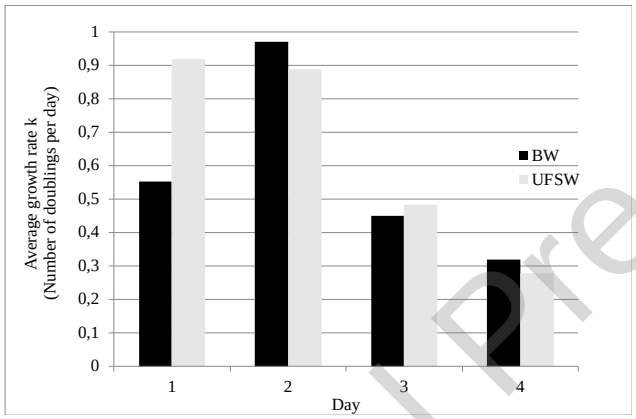
UFSW compared to BW from D0 to D4. UFSW exhibits low turbidity, thus allowing better access to light.

It should be noted that a crop that grows at a rate of one division per day has a doubling time of one day and an instantaneous growth rate of 0.693 d^{-1}. The average growth rates of cultures in 2 L and 30 L (Figure 7) showed a greater growth in UFSW compared to in BW between D0 and D1, with 1.1 doubling d^{-1} for UFSW versus 0.83 doubling d^{-1} for BW in a 2 L flask and 0.92 and 0.55 doubling d^{-1} in a 30 L cylinder. This trend was reversed in 2 L; there was stronger crop growth in BW from D2 to D4 due to the constant value observed at D3 for UFSW in Figure 6. Growth stabilized in a 30 L cylinder, and growth was relatively similar at D3 and D4 between the two conditions due to a similar growth between D3 and D4 for UFSW and BW. This difference could be explained by (i) the nutrient solutions used for the cultures. Indeed, a modified Provasoli adapted to the BW quality was used for the BW condition, while a classical Conway solution was used for the UFSW condition. There was a possible limit of available nutrients in USFW, thus explaining the cessation of growth after D3. (ii) there is a natural presence of metals in BW, unlike in UFSW. In fact, metals, particularly copper and zinc, are more toxic than other contaminants, such as insecticides. These results can therefore be explained by the fact that metals have a direct action on microalgae, unlike insecticides. Microalgae have specific proteins that act as intracellular buffers of metals, such as phytochelatins[44]. These metals therefore undoubtedly affect cells, reflected by a delay in the start of growth in the presence of copper [45]. However, in a previous study [15], the cultivation of T-Iso in UFSW led to higher microalgae concentrations than in classical seawater (filtrations 5 µm, 1 µm, 0.22 µm, and UV treatment) under the same culture conditions and with the same nutrients added. To confirm this finding, similar experiments were conducted in an Erlenmeyer flask and a 2 L flask with purified (filtrations 10 µm, 1 µm, 0.22 µm) and autoclave sterilization seawater (ASW) and UFSW. Regardless of the initial concentration (1 or 2 Mcells mL^{-1}) in the Erlenmeyer, flask after 4 days, faster growth of cultures were observed on UFSW than on ASW (x1.7 versus x1.5). After transfer from the Erlenmeyer flask to a 2 L flask, similar results were observed. The growth rates were comparable, since 22 Mcells mL^{-1} even higher concentration values in UFSW are observed due to the higher concentration of T-iso in the Erlenmeyer flask after 4 days (i.e. before the transfer in 2 L flask at D0) (Figure 8). In addition, the variation in light exposure (µmol m^{-2} s^{-1}) versus the microalgae concentration was observed for UFSW and ASW (Figure 8) in a 2 L

flask. Regardless of the quality of water, light exposure was a function of the microalgae concentration.



(a)



(b)

Figure 7: Average growth rate k of T-Iso cultures in a 2 L flask (a) and 30 L cylinder (b).

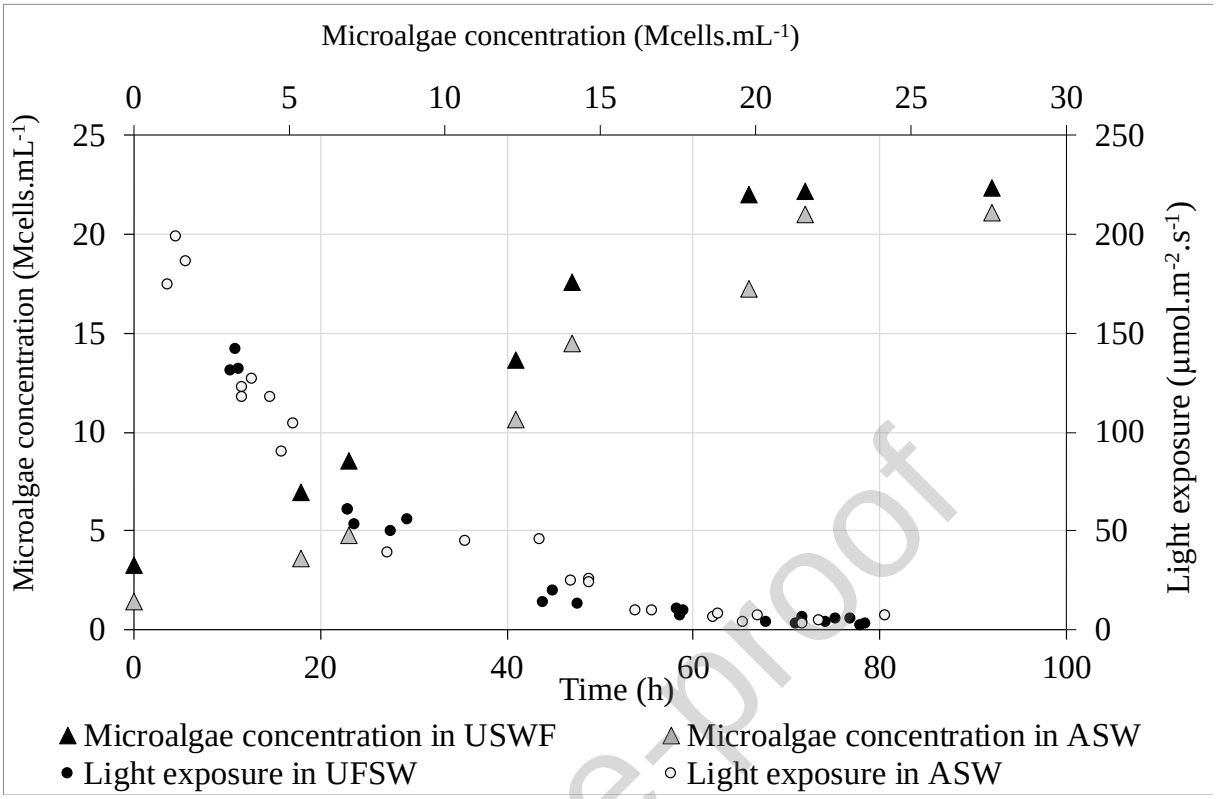


Figure 8: Variation of light exposure as a function of the microalgae concentration for different qualities of water in a 2 L flask [UFSW and ASW].

The concentration of cultures at D4 in 30 L cylinders is an important parameter concerning the yield of microalgae production. The average concentration of 30 L cylinders was calculated using the concentrations of 38 cylinders cultivated in UFSW and 43 in BW. A significantly higher concentration of the order of 1.4 Mcells mL^{-1} was obtained for UFSW (10.6 Mcells mL^{-1}) compared to BW (9.2 Mcells mL^{-1}) (Student's *t*-test, $\alpha = 0.05$, p-value = 0.002), demonstrating an increase of 15.5%.

Different aspects of the T-Iso culture were studied: appearance, mobility, effective use, growth, concentration, and contamination of cultures under conditions of BW commonly used in production at VN and UFSW using an UF pilot plant. T-Iso cultures showed greater growth in UFSW than in BW in 30 L cylinders throughout the culture. Because cultures in UFSW reach a constant value faster than BW, the values at D3 were compared. As culture transplantation should always be done in the exponential phase, it is likely that the 4-day cycle is no longer suitable for growing in UFSW and could potentially be reduced by one day, resulting in a 25% time reduction compared to BW. The study of the daily growth of the

cultures confirmed faster growth in UFSW during the first 24 h, and the growth rate was equal to that of the culture in BW on D2. The final concentration in 30 L cylinders on D4 was greater in UFSW (+ 15.5%) than in BW, although the two average concentrations remain acceptable for production conditions. Finally, less contamination was observed in UFSW cultures in 30 L cylinders compared to BW cultures, although a greater amount of contamination was observed in small volumes (Erlenmeyer and 2 L flasks) in UFSW. This difference in contamination between large and small volumes of BW can be explained by the fact that small volumes of BW are boiled, whereas larger volumes of BW cannot be boiled. The greater contamination of small volumes of UFSW remained negligible compared to a reduction of more than 30% in the rate of contamination of the 30 L cylinders, which is a significant advantage of UFSW. To explain the presence of ciliates in UFSW, we hypothesized that contamination occurs during manipulation or cleaning of containers. Because a contaminated container is not used for transplanting or feeding, it is preferable to lose small containers, which are often present in many replicates, rather than 30 L or 300 L cylinders. Despite the fact that 6–13% contamination was observed in small volumes, the general appearance of cultures on UFSW showed better stabilization, potentially easing the storage and preservation of small strains. The growth rate appears to be inversely proportional to the concentration of UFSW. Predictions may be possible with a greater number of results. With BW, the results are more random, and no prediction was possible.

3.3. Hatching of *C. gigas*

Temperature, salinity, and pH were measured in larval jars of seawater and UFSW before each cross. The average water temperature before crossing was 24.7 ± 0.6 °C for SW and 24.8 ± 0.4 °C for UFSW. The mean salinity was 36.2 ± 0.8 ppm for SW and 36.3 ± 0.6 ppm for UFSW. Finally, the average pH was 8.7 ± 0.1 for SW for UFSW. For each of the crossings, the parameters in the two media were similar. If the 2N hatching rate was good in SW (> 35%), then it was identical in UFSW (Figure 9). However, if it appeared worse, the hatching rate was improved using UFSW with hatching doublings (test 6). 3N hatching is more difficult to obtain than 2N because the tetraploid sperm used for 3N are less mobile, and therefore less fertile, than 2N sperm [46]. The results were very heterogeneous, with an improvement in one experiment using UFSW (300%) and three experiments using SW; SW had an average hatching rate of 15–20%, while UFSW had an average of 5%. These experiments were carried out in special weather conditions (heat waves and strong

thunderstorms), during which the UF pilot plant operated under extremely critical conditions of abnormally low production flow caused by significant fouling. These results therefore require confirmation using other tests.

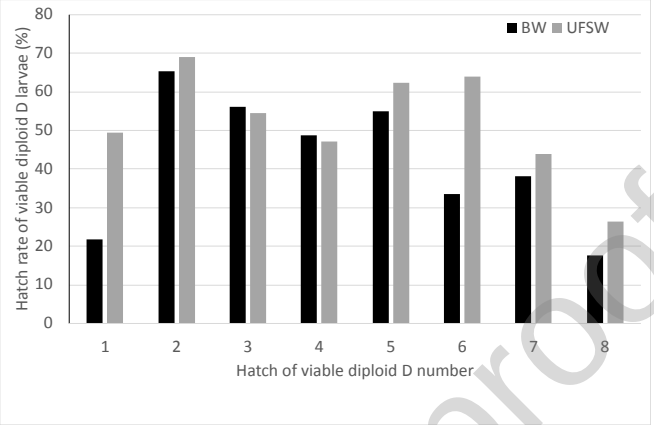


Figure 9: Hatch rate of viable diploid D larvae in UFSW and BW.

During the experiments, so-called “favorable” or “critical” periods for fertilization were identified due to the production results in an industrial hatchery. Both diploid and triploid fertilization results were classified in terms of hatch rate and larval abnormality. The abnormality rates and viable larval hatch rates during each period and for each ploidy were calculated (Figure 10). Similar abnormality rates were observed between SW and UFSW on diploid crosses during the favorable and critical periods (Figure 10). The rate of abnormality during the favorable period was identical for triploid crosses, while it was higher during the critical period (80% in UFSW against 50% in SW). The hatch results of the critical triploid crosses are important for analyzing the utility of the UFSW pilot plant and will need to be supplemented with a further series of tests.

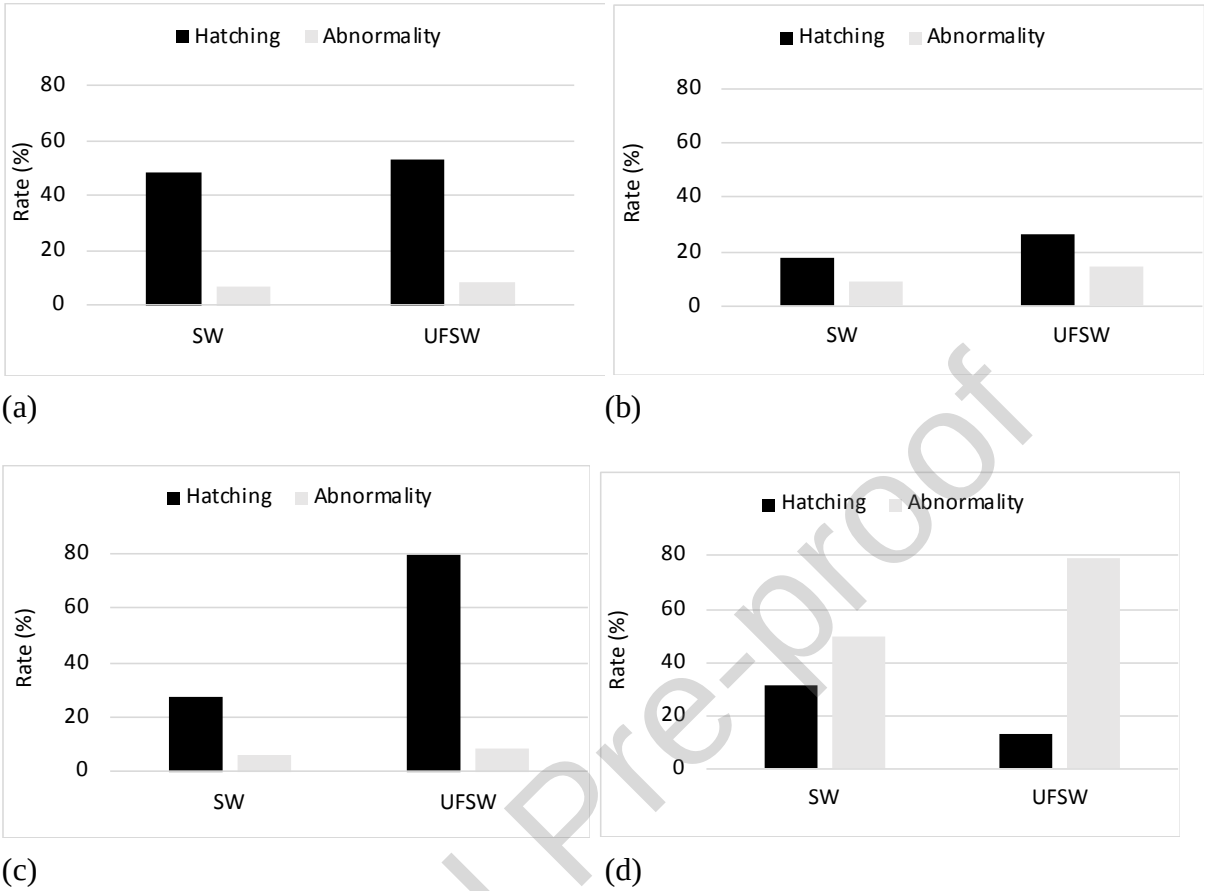


Figure 10: Hatching and abnormality rate of diploid (a, b) triploid (c, d) crosses during the favorable (a, c) and critical period (b, d) in SW and UFSW.

The results of bacteriological monitoring are presented in Figure 11. A fluctuation in the bacterial load (total flora) was observed in both SW and UFSW, without correlation. A comparison of these data with those obtained by Cordier *et al.* (2020) [30] shows that the values obtained at the output of UF are very high; indeed, bacterial loads lower than 500–1,000 CFU mL^{-1} were observed in previous experiments. Only the bacterial load in UFSW at the start of the manipulations, with a value of 4.8×10^2 CFU mL^{-1}, was in agreement with the previous study [30]. We can assume that contamination occurred at the outlet of the UF pilot plant, which seems to show a strong decrease in bacterial load after cleaning. Measurements of *Vibrio* and total flora at the outlet of the UF membranes made it possible to highlight the non-detection of *Vibrio* in the permeates. A 3.5 Log reduction and a total flora content of less than 80 CFU mL^{-1} were observed when the upstream concentration was measured at 20,000 CFU mL^{-1}. These good retentions at the exit of the membranes can only be maintained in the rest of the installation with regular maintenance.

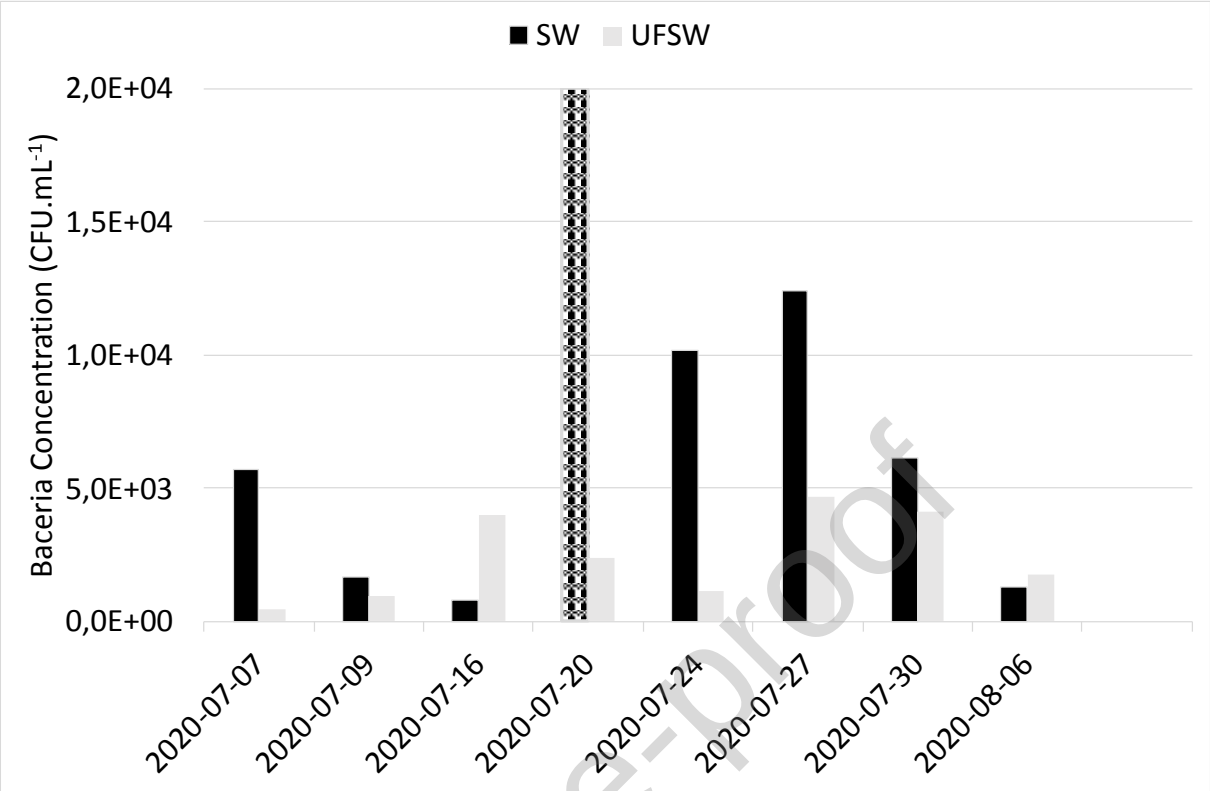


Figure 11: Variation of the bacterial concentration in the water of the jars crossed by SW and UFSW and the uncountable bacterial load (column grid).

The use of UFSW for outbreaks addresses the issue of fluctuating hatch rates and mortality in farms. This study made it possible to compare the efficiency of the UF pilot plant with the filtration and disinfection processes commonly used in hatcheries. During the experiments, two periods were identified: a favorable period for breeding (in July) and a critical period (in August). These periods are determined by the results of very low fertilization rates and/or a high rate of abnormality, not only in the R&D department, but also in the production hatchery of VN. This study showed that in a good period, the hatching rates of diploid crosses were similar between UFSW and SW and the abnormality rate remain acceptable compared to industry standards. Regarding the triploid crosses, only one fertilization was carried out during this period, and the results of this unique crossing showed a strong improvement in UFSW, as confirmed in the production department. In a critical period, the hatching results of triploid fertilizations were superior in SW compared to in UFSW, with a 40% higher abnormality rate observed in UFSW. Triploid crossing is generally a more difficult cross to achieve than diploid crossing, which is why acceptable hatch rates in production are in the range of 25–35% for triploids and 35–45% for diploids.

4. Conclusion

The installation of an UF pilot plant at the hatchery scale may be of interest for the culture of T-Iso. In fact, switching from culture in BW to culture in UFSW significantly reduces the risk of contamination; in addition, unlike BW, it is not necessary to boil UFSW. UFSW culture also shows faster growth with a shorter exponential growth phase, and UFSW exhibits a low turbidity allowing better access to light; these features allow a reduction in growth cycles and limit the glassware and daily transplanting time. The efficiency of UFSW for fertilization of *C. gigas* has not been clearly demonstrated. When the external environmental conditions are favorable, the pilot plant makes it possible to obtain hatching rates similar to or even higher than those obtained in 1-µm filtered and UV-disinfected SW: thus, a normalization with UFSW seems to take place. In the so-called "critical" period, the UF pilot plant does not achieve higher hatch rates. The stabilization of hatch rates expected using UF was not achieved. Therefore, the quality of seawater for this type of crossing is a determining factor of culture. The main objective of installing the UF pilot plant was to improve hatch rates during critical times and on this type of crossing. However, the use of the UF pilot plant did not seem to improve hatching rates and tended to increase the abnormal rate during triploid fertilization. We therefore hypothesized that this high abnormality result, which is synonymous with degradation of the seawater quality was due to accumulation on the membrane or release of toxic elements in UFSW. This problem of seawater quality can be localized and extended to the whole of the Polder, is not explained by recording the physicochemical parameters and is only identified downstream of fertilization. This may be due to the presence of chemical contaminants in the water, which may be linked to the proximity between the agricultural and shellfish polder, but also to the flows of continental waters [47]. These compounds are not retained by the membrane because of their very low molecular mass (pesticides or toxins of microalgae). In addition, the evolution of the bacterial load suggests that the pilot plant was more efficient at the start of the experiments. The data collected by the pilot plant confirmed a very strong fouling of the membranes at the beginning of August, which is a period of heat waves and thunderstorms. These conditions could "stress" microalgae present in the water and a rejection of exopolysaccharides, which are well known to strongly foul membranes, can occur.

These experiments highlight the importance of (i) maintenance of the UF process, (ii) periods of the year that are not favorable for oyster fertilization for both water treatments

studied, and (iii) better fouling control to optimize the production of treated water in terms of quantity and quality. For this last point, the use of a pretreatment such as sand filtration before UF seems to present a solution, guaranteeing the stability of the process and thus of the water produced. In conclusion, this study highlighted that UF could be used in hatcheries/nurseries for water treatment. Indeed, this process produces water with a quality adapted to the culture of microalgae and oyster fecundation. Although more experiments are needed, UF presents different advantages: the possibility of implementing one process for different applications in shellfish culture and the reduction of treatment steps and parallel lines of water production, which lead to advantages in terms of time, materials, and maintenance. With an optimized pretreatment and maintenance process, UF may present an efficient solution to secure shellfish industrial facilities.

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CRediT authorship contribution statement

T. Eljaddi : acquisition of data, carried out the experiment, analysis and/or interpretation of data, drafting the manuscript
S. Ragueneau : acquisition of data, carried out the experiment
C. Cordier : analysis and/or interpretation of data, Drafting the manuscript
A. Lange : analysis and/or interpretation of data, Conception and design of study, planning and supervised the work,
M. Rabiller : acquisition of data, analysis and/or interpretation of data

C. Stavrakakis : Drafting the manuscript, Conception and design of study, planning and supervised the work
P. Moulin : Drafting the manuscript, Conception and design of study, Project manager

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: