Effect of different culture media on the cells growth of Isochrysis galbana, Tetraselmis suecica and Chaetoceros calcitrans

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Abstract

The aim of the present study was to determine the optimal culture medium for the growth of *Isochrysis galbana, Tetraselmis suecica* and *Chaetoceros calcitrans*. These microalgae were cultured in three replicates using seven different culture media, including commonly used agricultural fertilizers in hatcheries. The study was conducted in 20 L polycarbonate aquariums, with 15 L of culture under standard experimental conditions of temperature, light intensity, photoperiod, and salinity. The performance of the microalgae was evaluated based on various parameters such as cell concentration, growth rate, division rate, doubling time, cell biovolume, and pH. The results showed that different culture media significantly affected ($p < 0.05$) the cell concentration of the microalgae. The culture medium based on Kw-21 liquid fertilizer produced the best cell concentrations for *Isochrysis galbana* and *Chaetoceros calcitrans*, while the culture medium based on CE fertilizer was the most suitable for *Tetraselmis suecica*. Other parameters were also significantly affected ($p < 0.05$) by the experimental factors, except for cell biovolume in *Tetraselmis suecica* at the stationary phase. The study suggests that using agricultural fertilizers is a viable and cost-effective alternative for mass production of microalgae.

Introduction

In Morocco, aquaculture with its three sectors (shellfish farming, fish farming and algae farming) is one of the sectors of the future in terms of food security, reinforced by several projects and agreements aimed at developing this sector. The shellfish farming sector is a main component of national aquaculture, represents 52% of total aquaculture production in 2020. Where microalgae are one of the fundamental constituents of any bivalve hatchery operation and considered the best food source for early instar larvae (Elias et al. 2003). Therefore, their production in good quantity and quality is obligatory to optimize the production process of larval and spat.

Microalgae are eukaryotic aquatic microorganisms, with very high photosynthetic efficiency, capable of producing valuable compounds such as proteins, carbohydrates, lipids and pigments (Banerjee et al. 2002; Tran et al. 2009; Hidalgo et al. 2013), moreover, they have a rapid and high reproduction rate. In hatcheries, live microalgae are used as food sources for all stages of bivalve growth, especially the early stages, due to their adequate nutritional compositions (Brown et al. 1997; Chowdhury et al. 2006).

For a better productivity and a high biomass concentration, microalgae produced in hatcheries need light intensity, photoperiod, temperature, pH, salinity, CO_2 concentration and the quantity and quality of nutrients or the culture medium. The latter is considered one of the essential elements to produce an excellent biomass and it represents all the nutrients in which microalgae can grow and multiply. Moreover, the mixture also increases the exchanges of nutrients and metabolites between the microalgal cells and the culture medium, and therefore have a high biomass yield. Also the nutrient compositions and the concentrations of culture media influence the production of algal biomass as indicated in a study realized by Choix et al. (2017) who showed the effect of compositions and concentrations; mainly

microelements and vitamins; of culture media on the production of biomass in the strain *Scenedesmus obliquus U169*. The culture of diatoms requires the presence of silicates in the culture medium.

However, some microalgae present a higher growth rate in one type of culture medium compared to another, this is in agreement with a study by Praba et al. (2016) who concluded that each of the microalgae has its own choice for culture medium for maximum growth. In this context, this study aims on the one hand, to determine the optimal environment for the growth of three species of microalgae, these are *Isochrysis galbana, Tetraselmis suecica* and *Chaetoceros calcitrans* which are very widely used in the Shellfish Station in Amsa for rearing larval stages, spat and broodstock because they are easy to cultivate and have a high nutritional value, in order to control and optimize the production of these species. On the other hand, to assess the efficacy of expensive commercial culture media, in comparison with alternative low cost culture media "fertilizer". Because the high cost of nutrient media for mass culture is major obstacle for hatcheries to maintain their production (Edwards 2005), and according to FAO (2004), the production of microalgae represents more than 40% of the overall cost of hatchery spat production, up to a shell length of the order of 5 mm.

A number of studies in different fields have shown the influence of culture media on the growth of several microalgae, for example *Tetraselmis sp., Chaetoceros sp., Chlorella sp., Nannochloropsis sp.* and *Dunaliella sp. (*Praba et al. 2016), *Dunaliella sp*. (Charioui et al. 2015; Allayban et al. 2020), *Chlorella sorokiniana* (Ribeiro et al. 2019), *Pseudopiediastrum boryanum var longicorne* (Sutkowy et al. 2018), *Scenedesmus obliquus U169* (Choix et al. 2017), *Scenedesmus obliquus* and *Micractinium reisseri* (Abomohra et al. 2016), *Skeletonema sp*. (Endar et al. 2012).

This study aims to carry out a study on the effect of each type of culture medium on the different parameters of cell growth of the three species of microalgae studied, in order to obtain the highest productivity at the lowest production cost.

Materials and methods

The present study was conducted at the Phytoplankton unit of the Shellfish Station at Amsa (SCA) under the Specialized Center in Zootechnics and Marine Aquaculture Engineering at M'diq (CSZIAM) of the National Institute of Fisheries Research (INRH), Tetouan, Morocco. The study focused on three commercially important microalgae species that are commonly used in shellfish hatcheries: *Isochrysis galbana, Tetraselmis suecica*, and *Chaetoceros calcitrans*. Various culture media, including agricultural fertilizers (both liquid and solid), were chosen for maintaining the inoculum and conducting the experiments. The selected culture media were F/2 (Guillard 1975), Conway (Walne 1966), Kw-21, MAP, NPK, NP, and CE, with their respective compositions detailed in Table 1.

Table 1

Composition of different media used for the cultivation of Isochrysis galbana, Tetraselmis suecica and Chaetoceros calcitrans

Chemical compounds	Concentration ($g.L^{-1}$)						
	F/2	Conway	Kw-21	MAP	NPK	NP	CE
$NaNO_3$	75.00	100	-	-	-	-	-
$NaH_2PO_4.H_2O$	5.00	26	-	-	-	-	-
Na_2EDTA	4.36	49.8	+	-	-	-	-
$FeCl_3.6H_2O$	3.50	1.3	-	-	-	-	-
$CuSO_4.5H_2O$	9.80	0.020	-	-	-	-	-
$ZnSO_4.7H_2O$	22.00	-	+	-	-	-	-
$MnCl_2.4H_2O$	180.00	0.36	+	-	-	-	0.014%
$Na_2MoO_4.2H_2O$	6.30	-	-	-	-	-	-
$CoCl_2.6H_2O$	10.00	0.020	+	-	-	-	-
Thiamine HCl	0.020	0.20	+	-	-	-	-
Cyanocobalamin	0.001	0.01	+	-	-	-	-
Biotin	0.001	-	+	-	-	-	-
$Na_2SiO_3.9H_2O$	30.00	-	-	-	-	-	-
H_3BO_3	-	33.6	-	-	-	-	-
$ZnCl_2$	-	0.021	-	-	-	-	-
$(NH_4)_6Mo_7O_{24}.4H_2O$	-	0.009	-	-	-	-	-
N_2	-	-	45	-	-	-	-
H_3PO_4	-	-	4	-	-	-	-
Amino acid complex	-	-	+	-	-	-	-
Fe^{2+}	-	-	+	-	-	-	-
$H_2BO_3^-$	-	-	+	-	-	-	-
N-total	-	-	-	4.2	-	-	-

Chemical compounds	Concentration ($g.L^{-1}$)						
	F/2	Conway	Kw-21	MAP	NPK	NP	CE
$N\text{-}NH_4$	-	-	-	4.2	-	-	-
P_2O_5	-	-	-	21.35	-	-	6%
P	-	-	-	9.31	-	-	-
NH_4^+	-	-	-	-	2	-	2.8%
$O_{10}P_4$	-	-	-	-	4	-	-
K_2O	-	-	-	-	4	-	-
$H_{16}N_5O_7P$	-	-	-	-	-	35	-
CON_2H_4	-	-	-	-	-	-	2.2%
NO_3^-	-	-	-	-	2	-	1.0%
k_2O	-	-	-	-	-	-	6%
Cu	-	-	-	-	-	-	0.002%
Fe	-	-	-	-	-	-	0.025%
Mn	-	-	-	-	-	-	0.014%
Mo	-	-	-	-	-	-	0.001%
Zn	-	-	-	-	-	-	0.004%

The study was conducted in 20 L transparent polycarbonate aquariums. These aquariums were sterilized and rinsed before being filled with 5 L of sea water, which was filtered at 0.2 µm and sterilized using UV. Fresh water was added to the sea water to achieve the desired salinity, and then the aquariums were enriched with the culture medium. The volumes of culture medium used for each aquarium were prepared according to the protocol described in the table provided below.

Table 2
Volumes used for the enrichment of each culture medium studied

Culture media	Volume added ($mL.L^{-1}$)
F/2	1
F/2*	0.1 or 0.2 (according to specie)
Conway	1
NPK	1
NP	1
Kw-21	0.5
MAP	1
CE	2

* Control medium

Afterwards, the aquariums are inoculated by the necessary volume of culture with an initial cell concentration of the order of 1.10^5 $cells.mL^{-1}$ at 25 ± 2 ºC under 10000 lux light with 12:12 hours light/dark cycle.

The start-up of the cultures is done directly after the inoculation step in FRP rectangular tank of 1500 L in order to put them in the desired conditions. The cultures are aerated by air diffusers to ensure their suspension.

Methodology adopted for monitoring the evolution of the three strains of microalgae

The Cell concentration (NC) was evaluated by daily counting using a Malassez counting chamber after immobilization of living cells with Lugol, under a binocular light microscope (x40 Magnification), the specific growth rate (μ) is calculated according to the equation used by Mohanadoss (2013), the division rate (K) per day and the doubling time (D) can also be calculated once the specific growth rate (μ) is known, for monitoring cell biovolume, biometric data is obtained in the laboratory using images captured by the camera of a Nikon Eclipse E200 microscope (Nikon Corporation, Tokyo, Japan) with the Nikon Ds-Fi2 digital camera at 40x magnification. This operation is done every two days and the processing of these images was carried out using image processing software (image J) which give the possibility to determine the dimensions of each microalgae cell, and to determine the pH use the pH-meter MM-43X.

All the tests and determination were performed in triplicate.

Statistical analysis

Statistical analysis and graphic illustrations were performed using the Microsoft Excel program. The data obtained were tested by ANOVA analysis of variance with significance level $p < 0.05$ using IBM-SPSS V23 software. In order to determine the significance of the treatments applied for the different species studied.

Results

The following figure presents the monitoring of the evolution of the cell concentration of the three species studied with different culture media (Fig. 1).

The culture of *Isochrysis galbana* enriched with Kw-21 medium was recorded a higher growth rate than the other media with a value of $6.85.10^6$ cells.mL^{-1} on the ninth day, for the other media, the maximum concentrations recorded represent about half of this recorded in Kw-21 medium (Fig. 1A). The best cell concentration results in *Chaetoceros calcitrans* were recorded in Conway ($4.5.10^6$ cells.mL^{-1}) and Kw-21 ($4.3.10^6$ cells.mL^{-1}) culture media (Fig. 1B). Concerning *Tetraselmis suecica*, the EC liquid fertilizer was presented the highest value of the cell concentration during the exponential phase with a value of $1.26.10^6$ cells.mL^{-1} (Fig. 1C).

The maximum growth rates were recorded in MAP medium (0.65 ± 0.06 day^{-1}) in *Chaetoceros calcitrans*, and in Kw-21 medium (0.50 ± 0.04 day^{-1}) in *Isochrysis galbana* while for *Tetraselmis suecica*, it was recorded in F/2 (0.1 mL.L^{-1}) control medium (0.28 ± 0.02 day^{-1}) (Fig. 2).

The maximum division rates were recorded in MAP culture medium in *Chaetoceros calcitrans* (0.94 ± 0.10 div.day^{-1}), and in Kw-21 medium in *Isochrysis galbana* (0.72 ± 0.06 div.day^{-1}) while in *Tetraselmis suecica*, the maximum value was recorded in F/2 (0.1 mL.L^{-1}) control medium (0.41 ± 0.03 div.day^{-1}) (Fig. 3).

Based on the results in Fig. 4, the minimum doubling time was recorded in MAP medium in *Chaetoceros calcitrans* (1.08 ± 0.11), and in Kw-21 medium in *Isochrysis galbana* (1.39 ± 0.11), while in *Tetraselmis suecica*, it was recorded in F/2 (0.1 mL.L^{-1}) control medium (2.46 ± 0.19).

The cell biovolume of the three microalgae species was measured during the exponential and stationary phases of the experiment, and the results are presented in Table 3. The highest values were recorded in CE medium for *Isochrysis galbana* (141.28 ± 14.45 µm^3) and *Chaetoceros calcitrans* (82.65 ± 8.6 µm^3) during the exponential phase, while for the stationary phase, the highest values were observed in F/2 (1 mL.L^{-1}) medium for these two species (110.02 ± 6.28 µm^3 and 125.76 ± 13.10 µm^3 respectively). For *Tetraselmis suecica*, the maximum values were recorded in NP medium for both phases of the experiment (483 ± 7.38 µm^3 and 409 ± 12.53 µm^3 respectively).

Table 3
Cell biovolume recorded during the stationary phase and the exponential phase in the species studied under the different culture media tested

Culture media	Cell biovolume (μm^3)					
	Isochrysis galbana		Chaetoceros calcitrans		Tetraselmis suecica	
	Ex	St	Ex	St	Ex	St
Conway	53.48 ± 8.83 d	84.33 ± 4.35 c	63.33 ± 12.6 ab	111.30 ± 12.71 ab	273 ± 13.12 cd	400 ± 10.27 a
NP	79.11 ± 4.05 bc	106.41 ± 7.76 ab	58.36 ± 16.8 b	90.14 ± 11.39 c	483 ± 7.38 a	409 ± 12.53 a
MAP	91.30 ± 11.20 b	63.12 ± 5.66 d	37.34 ± 9.59 cd	97.17 ± 7.80 bc	304 ± 1.98 cd	384 ± 8.56 a
NPK	94.77 ± 4.25 b	84.29 ± 3.29 c	36.81 ± 16.99 d	113.96 ± 12.19 a	295 ± 10.83 cd	370 ± 7.19 a
KW-21	61.01 ± 10.13 cd	98.82 ± 5.40 b	38.13 ± 13.23 cd	94.90 ± 13.78 c	276 ± 6.00 cd	378 ± 13.30 a
F/2 (1 $mL.L^{-1}$)	50.44 ± 10.61 d	110.02 ± 6.28 a	55.74 ± 13.8 bc	125.76 ± 13.10 a	326 ± 12.30 c	406 ± 9.84 a
F/2 (0.1 $mL.L^{-1}$)	***	***	50.89 ± 11.87 bcd	90.39 ± 7.07 c	399 ± 4.62 b	372 ± 20.58 a
F/2 (0.2 $mL.L^{-1}$)	60.48 ± 9.04 cd	98.59 ± 6.92 b	***	***	***	***
CE	141.28 ± 14.45 a	60.74 ± 8.99 d	82.65 ± 8.6 a	46.03 ± 10.55 d	249 ± 7.14 d	405 ± 16.19 a
With: a > b > c > d; Ex: exponential phase; St: stationary phase						

The results of the pH measurement showed that the CE medium was presented with the lowest pH values in the three species, on the other hand, the F/2 (1 $mL.L^{-1}$), NP and F/2 (0.2 $mL.L^{-1}$) media were presented the highest values of pH in *Isochrysis galbana*, while for *chaetoceros calcitrans* and *Tetraselmis suecica* the pH was more or less the same in all culture media (Table 4).

Table 4
pH values recorded at the end of the culture for each species studied under the different culture media tested

Culture media	pH of culture media		
	Isochrysis galbana	Chaetoceros calcitrans	Tetraselmis suecica
Conway	8.11 ± 0.00	8.45 ± 0.04	8.38 ± 0.23
NP	8.35 ± 0.03	8.45 ± 0.01	8.44 ± 0.01
MAP	8.17 ± 0.01	8.45 ± 0.01	8.42 ± 0.03
NPK	8.08 ± 0.04	8.29 ± 0.06	8.26 ± 0.09
KW-21	8.15 ± 0.04	8.47 ± 0.04	8.47 ± 0.01
F/2 (1 $mL.L^{-1}$)	8.48 ± 0.10	8.47 ± 0.10	8.44 ± 0.01
F/2 (0.1 $mL.L^{-1}$)	***	8.39 ± 0.11	8.32 ± 0.06
F/2 (0.2 $mL.L^{-1}$)	8.34 ± 0.01	***	***
CE	7.73 ± 0.02	7.73 ± 0.14	7.12 ± 0.22

Discussion

The culture medium is an essential factor in the process of microalgae production, it plays an important role in the completion of its growth cycle durations, and each species of these microalgae present its preferences against this factor. For example, diatoms are the only unicellular organisms to have a siliceous cell wall, consequently a contribution of silicate is essential for the growth of this group of diatoms, then the culture media must meet the nutritional requirements of the microalgae that we want to produce.

In the present study, the results obtained from the culture medium test in *Isochrysis galbana, Chaetoceros calcitrans* and *Tetraselmis suecica* show that the different culture media tested have a significant effect ($p < 0.05$) on the growth performance of these species, except in *Tetraselmis suecica*, where the cell biovolume shows no significant effect at the stationary phase.

Effect of culture media on growth performance of Isochrysis galbana

Regarding the cell concentration, this species recorded its maximum value of the cell concentration in Kw-21 liquid fertilizer on the 9th day of the test with a value more than twice that obtained in the other culture media, this medium is followed respectively by F/2 (1 $mL.L^{-1}$), Conway and F/2 (0.2 $mL.L^{-1}$) control, but it should be noted that under these last three culture media the difference between the values of the cell concentrations is not significant ($p > 0.05$). These results are in accordance with Panta Vélez Rodolfo Patricio et al. (2016) who noted that there are significant differences ($p < 0.05$) between the values of the

cell concentration obtained for the *Isochrysis galbana* under the influence of different culture medium treatments with the agricultural fertilizers used. On the other hand, the results show a low power of evolution of the cell concentration of *Isochrysis galbana* in MAP fertilizer compared to the other culture media, while in the culture medium enriched with CE, *Isochrysis galbana* did not evolve. It should be noted that during the study of Jbari et al. (2020), the cell concentration of *Isochrysis sp.* with MAP fertilizer was slightly higher than the control, this is different from what was found in our study, this can be explained by the effect of the difference between the N:P:K ratio, as well as the vitamins used in this study with those used in our study.

For the growth rate, the maximum value is recorded in the liquid fertilizer Kw-21, while the lowest values were recorded in F/2 (0.2 $mL.L^{-1}$) control, followed by CE which presented a low value of the growth rate, because with this condition of culture medium *Isochrysis galbana* did not evolve well. According to Panta Vélez Rodolfo Patricio et al. (2016) they found that in *Isochrysis galbana*, there are significant differences between the values of growth rate obtained under the effect of the different treatments with agricultural fertilizers compared to the control medium, but the values recorded in this latter treatment were lower than those obtained in our control medium, this can be explained by the effect of ammonium toxicity, as a high concentration of ammonium is toxic for *isochrysis galbana* (Kaplan et al. 1986), a study by Ezeani and Abu (2019) recorded low growth in *Chlorella* in the most nitrogen-concentrated culture medium. Different results were obtained with *Isochrysis aff. Galbana*, where the use of agricultural media based on fertilizers showed no significant effect of growth rate compared to the F/2 control medium, which shows that agricultural fertilizers are an appropriate alternative for the cultivation of microalgae (Valenzuela-Espinoza, Millan-Nunez and Nunez-Cebrero 2002).

Concerning the division rate, the maximum value is recorded in Kw-21, followed by MAP, NPK and NP respectively, the values of the latter three are higher than the value recorded in the control medium, while the lowest value is recorded in CE. These results are perfectly in agreement with a study done by Gonzalez et al. (1984) on the growth of four species of microalgae under the effect of the use of agricultural fertilizers as a nutrient source in the production of these microalgae, they found a significant difference ($p < 0.05$) between the values of the division rate obtained for *Isochrysis aff. Galbana* under the different treatments tested. For *Isochrysis sp.*, contradictory results indicate that there were no significant differences between the treatments with the commercial fertilizers used on the division rate (Pina et al. 2007).

About the doubling time, the best value in recorded in Kw-21, followed by MAP and NPK respectively. This result is consistent with that reported by Panta Velez Rodolfo Patricio et al. (2016) who reported that for *Isochrysis galbana*, there is a significant difference in the doubling time recorded under the influence of agricultural fertilizers, as well as the best values obtained were in the Gaillard F/2 medium, followed by a medium based on agricultural fertilizer. In contrast, Valenzuela-Espinoza, Millán-Núñez and Núñez-Cebrero (2002) report that for *Isochrysis aff. galbana* in guillard F/2 medium and the fertilizer tested, the values of the doubling time obtained are similar, no significant effect ($p > 0.05$), this can be explained by the notable difference between the conditions of temperature or light intensity applied to the culture

during their study compared to what was applied during our study. Because according to Renaud et al. (2002) and Kaplan et al. (1986) reported that the optimal temperature for the growth of *Isochrysis galbana* is 25°C and 27°C respectively, whereas Valenzuela-Espinoza, Millán-Núñez and Núñez-Cebrero (2002) used a constant temperature (20 ± 1°C).

For the cell biovolume, and during the exponential phase, *Isochrysis galbana* recorded its maximum value in the culture medium enriched with CE, followed by NPK and MAP respectively, while during the stationary phase the maximum value is recorded in F/2 (1 $mL.L^{-1}$), followed by Kw-21 and the control medium.

In the scientific literature, despite the extensive studies in the field of microalgae production and in the determination of optimal conditions for the cultivation of different species, few are those based on cell biovolume to determine these conditions and those studying the effect of different types of culture medium. Although it was not found in the literature studies of the same theme to compare the values of cell biovolume obtained in the study.

Effect of culture media on growth performance of Chaetoceros calcitrans

For cell concentration, it seems that the best medium for the culture of *Chaetoceros calcitrans* is the culture medium enriched by Conway, followed respectively by Kw-21 and NPK. These results are identical to the one reported by Chowdhury et al. (2008) who tested the culture of *Chaetoceros affinis* in several culture media including NPK, and they proved that there is a significant difference between the means recorded in each treatment. Also for Lestari, Mukhlis and Priyono (2019), who found that the application of Kw-21 + Si fertilizer and another nutrient fertilizer had a significant effect on the growth of *Chaetoceros calcitrans*, they also found that under the Kw-21 + Si treatment the cell concentration is much higher than the other treatments. This is probably due to the silicate concentration added in the Kw-21 fertilizer which is still optimal for the growth of this diatom species. Knowing that the silicate is one of the most important components in the life of diatoms, as they use silicates in very large quantities ideal for the formation of external frustules (Aprimara 2010), in order to have a high resistance to environmental stresses. On the other hand, contradictory results were reported by Pacheco-Vega and Sánchez-Saavedra (2009) who showed that there was no significant difference between F/2 and NPK in terms of cell concentration for *Chaetoceros*. This contradiction may be due to the fact that the latter used NPK whose N and P concentrations are identical to that in F/2 while the NPK used in our study contains high concentrations of N and P compared to F/2. The lowest value is observed in CE, this could be explained by the effect of pH, since the CE medium presented low pH values compared to other culture media, since the beginning of the study, as Suriawiria (2005) pointed out, lowering the pH of the culture medium affects the metabolism and growth of microalgae by altering the nutritional balance.

About the growth rate, it recorded its maximum value in MAP, followed by NPK and NP respectively, while it recorded its minimum value in F/2 (0.1 $mL.L^{-1}$) control medium. Similar results were obtained by Lestari, Mukhlis and Priyono (2019) who found that the application of nutrient fertilizer has a significant

effect on the growth rate of *Chaetoceros calcitrans* and also by Piña et al. (2007) with *Chaetoceros muelleri*.

The best optimal doubling time is recorded in MAP. This result is inconsistent with the results of Lestari, Mukhlis and Priyono (2019) who found that the self-duplication time of *Chaetoceros calcitrans* is faster in Kw-21 + Si medium compared to other media. According to Utomo et al. (2005) the doubling time is influenced by biological factors such as body shape and nature and non-biological factors such as nutrients, temperature and light. In our case, the temperature and the light are standard in all cultures, so the contradiction may be due to the shape and nature of the body or other nutrients such as nitrogen or phosphorus.

Regarding, the division rate, the *Chaetoceros calcitrans* recorded its maximum value in the culture medium enriched by MAP, followed by NPK, NP and Conway respectively, while it recorded its minimum value in the F/2 (0.1 $mL.L^{-1}$) control. This is in perfect agreement with the result obtained by Piña et al. (2007), they showed that there is a positive trend under the influence of urea and another nutrient fertilizer compared to the Guillard F/2 medium for the division rate of a diatom species, namely *Chaetoceros sp.*

During the exponential phase, the maximum value of cell biovolume is recorded in CE, followed by Conway and NP respectively, while during the stationary phase the maximum value is recorded in F/2 (1 $mL.L^{-1}$), followed respectively by NPK and Conway. Although it was not found in the literature studies on the same subject to compare the values of cell biovolume obtained in the study, it is important to mention that fertilizers like NPK, Kw-21 and MAP had a positive effect on cell biovolume at the end of the study.

Effect of culture media on growth performance of Tetraselmis suecica

About the cell concentration of *Tetraselmis suecica*, the best result is observed in CE, this may be due to the high concentration of N, P and vitamins provided by this culture medium used, this hypothesis was shown by Dammak et al. (2017) who linked the high production in biomass for *Tetraselmis* to the high concentration of N. On the other hand, the low value is recorded in MAP, with means inferior to the control. This result is contradictory to the one reported by Jbari et al. (2020) who tested the cultivation of *Tetraslmis* genus species under the influence of three types of fertilizers including MAP, and proved that the treatment in MAP had no noticeable effect on the growth of *Tetraslmis sp.* in comparison with the control medium F/2. In the scientific literature, there are works related to the use of agricultural fertilizers in the production of microalgae for aquaculture with contradictory results to what we have obtained as Piña et al. (2007), which tested the cultivation of four types of microalgae including *Tetraselmis suecica* in several culture media with different agricultural fertilizers and proved that there was no difference between the treatments used. Can be explained on the one hand, by the difference in volume of the culture medium which was 3 L against 15 L in the study we conducted, in effects in another study under the influence of three types of fertilizers for *Tetraslmis sp.*, the volume of the culture medium was 250 mL, the analysis indicated no significant effect on the production potential in MAP and TSP compared to the control medium (Jbari et al. 2020).

The *Tetraselmis suecica* recorded a high growth rate in Kw-21, followed by the F2 (0.1 $mL.L^{-1}$) control and CE respectively, as well as there is no clear difference between the values of the maximum growth rate in these last culture media. While it recorded its low value in MAP. This result is quite coherent with the one reported by Jbari et al. (2020) that found that for *Tetraselmis sp*. the different fertilizers tested, included the MAP fertilizer, they had an effect on the growth rate, knowing that in MAP the crop presented a low value similar to that obtained in our study.

About the doubling time, it recorded its minimum value in the control medium F/2 (0.1 $mL.L^{-1}$), followed by Kw-21 and CE, respectively, it should be noted that for the latter culture media, the difference between the generation rate values is not significant. For *Tetraselmis suecica*, despite the extensive studies in the field of microalgal production improvement, few of them are based on doubling time to determine the optimal conditions for production. On the other hand, for other types of flagellate species, when studying the influence of two types of fertilizers on *Isochrysis galbana*, in comparison with the control medium F/2, they reported that there is a significant difference observed in the duplication time under both culture media of this microalgae (Panta Vélez Rodolfo Patricio et al. 2016).

Regarding the division rate, the maximum value is recorded in the control medium F/2 (0.1 $mL.L^{-1}$), followed by CE and Kw-21 respectively, while it recorded its minimum value in MAP. These results are contradictory with that reported by Piña et al. (2007), they found that for the *Tetraselmis* suecica, there was no significant effect of culture medium on the division rate.

During the exponential phase, the maximum value of cell biovolume is recorded in NP, followed by the F/2 (0.1 $mL.L^{-1}$) medium, and the minimum value is recorded in CE. On the other hand, during the stationary phase, the difference between the cell biovolume values obtained in most of the culture medium conditions is not significant ($p > 0.05$). In addition, the maximum value is recorded under the same culture medium during both phases of the study. These results are similar with the results of Candido and Lombardi (2020) who found that there is a significant effect of different culture medium treatments on cell biovolume for several microalgal species including *Tetraselmis sp*..

Conclusion

The main objective of this study is to improve the mass production of three species of microalgae of shellfish interest. Thus, the effects of different culture media, including agricultural fertilizers, on the growth performance of these species were studied.

The results obtained in terms of cell concentration showed that for mass production, the growth of these species is influenced by the factors studied, and the best growth performance is obtained in: *Isochrysis galbana* in a culture medium based on Kw-21 liquid fertilizer, *Chaetoceros calcitrans* in a culture medium based on Kw-21 liquid fertilizer and Conway's medium, whose averages are statistically equal, and *Tetraselmis suecica* in a culture medium based on CE liquid fertilizer.

In addition, in terms of growth rate, division rate, doubling time, and cell biovolume, it generally turned out that under the conditions tested, the studied factors have a significant effect ($p < 0.05$) on these different parameters. With the exception of *Tetraselmis suecica* at the end of the test (stationary phase), where the cell biovolume was not affected by treatments under different culture media.

Therefore, the results obtained from the studies carried out can contribute to improving the culture and productivity of microalgae at the Shellfish Station in Amsa, as well as reducing production costs. Since it is obvious that for the microalgae species studied, agricultural fertilizers are easily assimilated, quite well than the complex media, which have a high cost. In this sense, the use of agricultural fertilizers as a culture medium becomes more practical.

Declarations

Disclosure statement: Conflict of Interest: There are no conflicts of interest.

Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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Figures

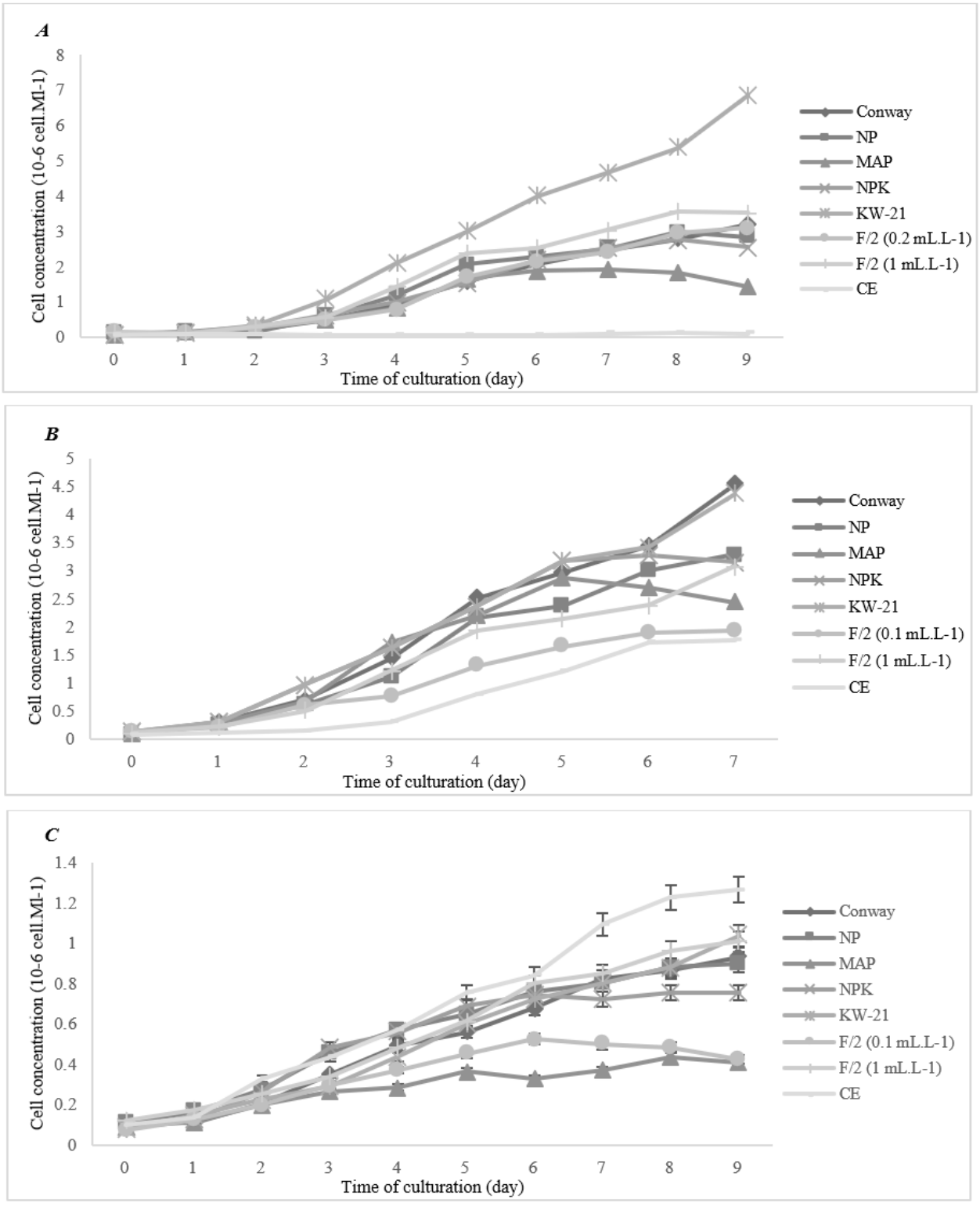

Figure 1

Evolution of the cell concentration of A. *Isochrysis galbana,* B. *Chaetoceros calcitrans* and C. *Tetraselmis suecica* with different culture media

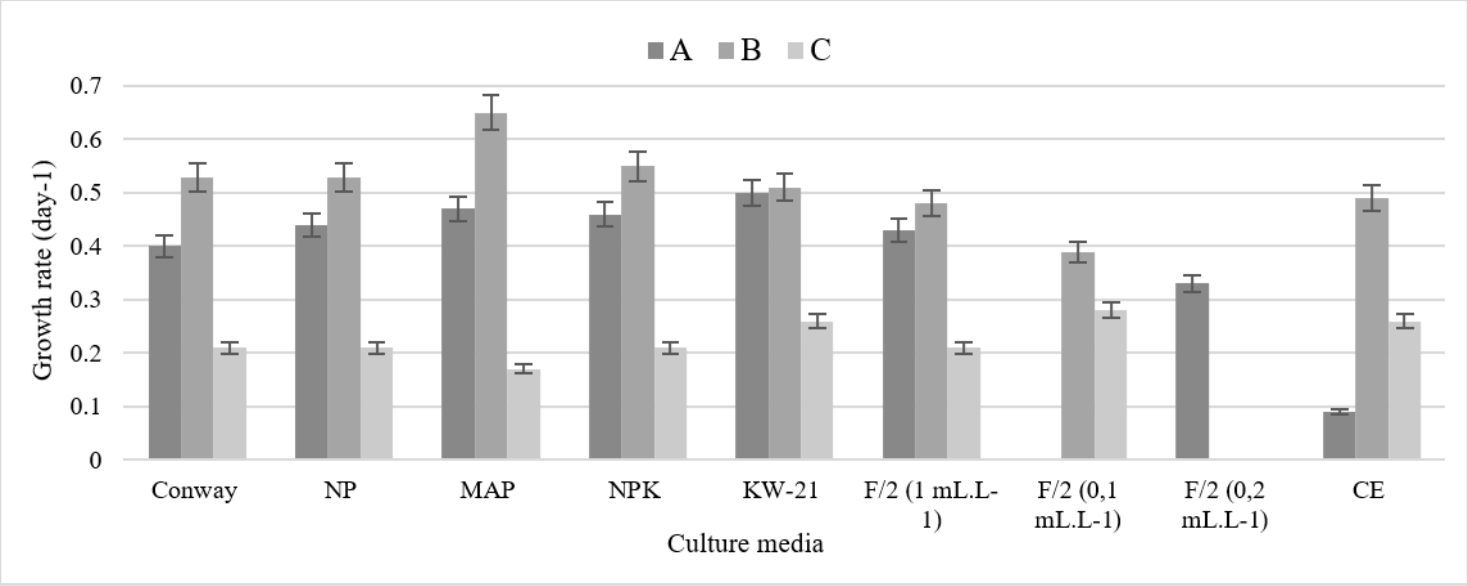

Figure 2

Evolution of the growth rate of A. *Isochrysis galbana,* B. *Chaetoceros calcitrans* and C. *Tetraselmis suecica* in different culture media

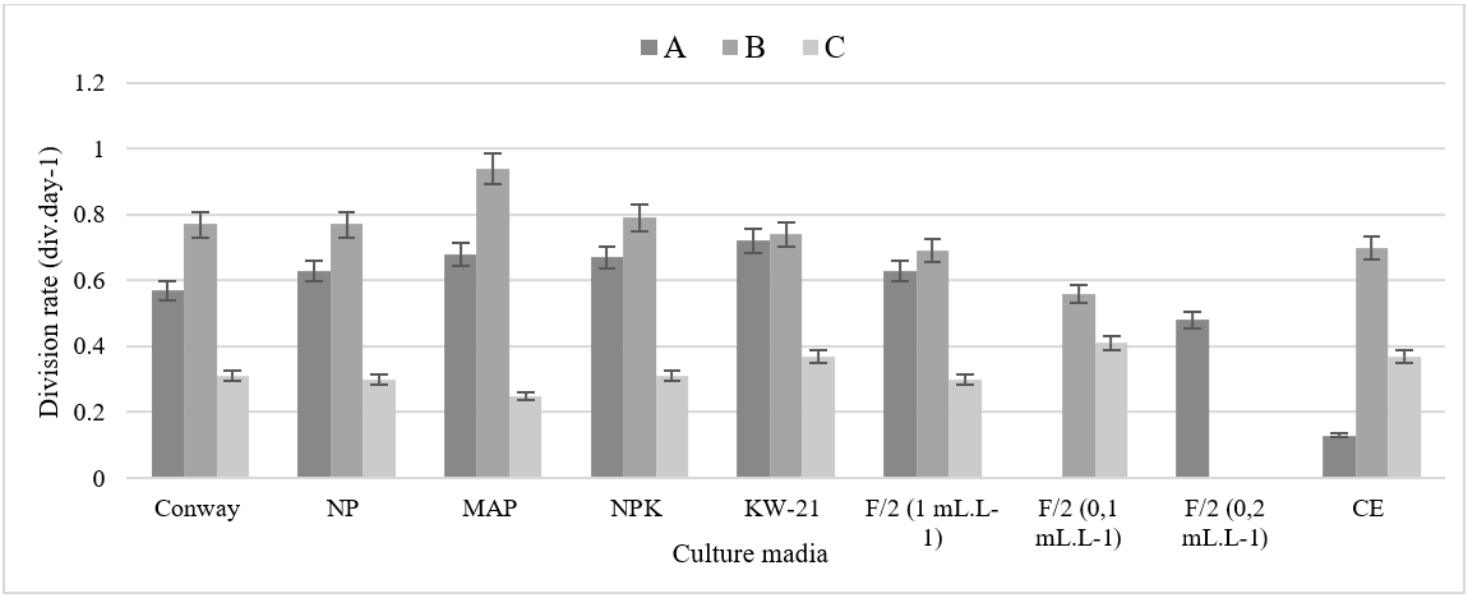

Figure 3

Evolution of the division rate of A. *Isochrysis galbana,* B. *Chaetoceros calcitrans* and C. *Tetraselmis suecica* in different culture media

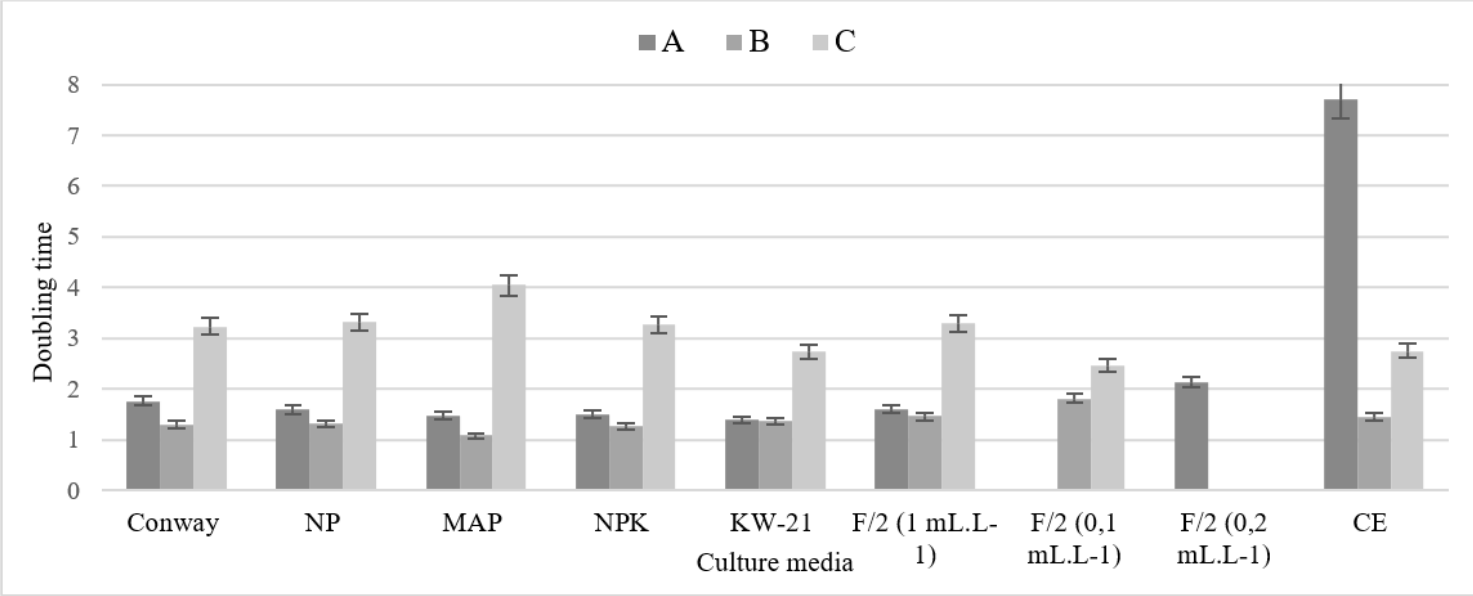

Figure 4

Evolution of the doubling time of A. *Isochrysis galbana,* B. *Chaetoceros calcitrans* and C. *Tetraselmis suecica* in different culture media