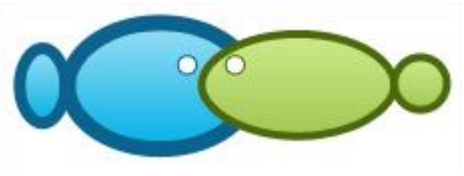

Isolation and characterization of potential microalgae strains from Moroccan coasts for their utilization as feed in shellfish hatcheries

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Abstract. Six local microalgal strains, recommended for nutritional programs in aquaculture, have been isolated along the Moroccan coasts. These strains were selected and characterized according to their size, growth performance and nutritional profile in terms of lipid, fatty acid and protein composition. The results of this study have shown an important growth performance of these strains under optimal conditions. The highest growth rate was observed for *Chaetoceros* sp. (0.80 d^{-1}) and the lowest one for *Nannochloropsis* sp. (0.20 d^{-1}). The average size and biovolume of the strains range from 2.57 to 12.83 (µm) and from 8.87 to 480.53 (μm^3) respectively, which appears to be suitable for nutrition purposes in shellfish pratices. Biochemical analysis provided a clear insight into the important nutritional profile of the local strains with high richness in terms of lipids and proteins content respectively (26 to 57% and 14 to 34%). Polyunsaturated fatty acids are consistently the phenotypic footprints for mariculture potential of microalgae. Eight components of polyunsaturated fatty acids were detected in this study where α-linolenic acid and eicosapentaenoic acid were communly encountred in the majority of strains. Thus, the microalgae isolated in this work, can be used alone or as part of a mixed diet in nutrition programs inside shellfish hatcheries.

Key Words: microalgae, isolation, growth kinetics, biochemical composition, shellfish nutrition, shellfish Hatcheries.

Introduction. Microalgae are a fast growing organisms with an exceptional metabolic plasticity (Rastoin & Kelly 2016; Zachleder et al 2016) that allows them to synthesize substances with high value-added. These organisms represent an interesting field for reserach and new promising bio-resource in several applications (Richmond 2003; Levine 2018). In aquaculture, these organisms can be used as main food for bivalve molluscs throughout their life cycle (Muller-Feuga et al 2007). For crustaceans and some fish species, microalgae are generally used either to feed larval stages or indirectly during zooplankton production (Muller-Feuga et al 2007; Richmond 2003). In shellfish farming, the production of microalgae is one of the key elements inside the hatchery and represent almost 40% of the overall cost of spat production (Helm et al 2006). In general, microalgae constitute a basic diet for bivalves which cannot be easily substituted by artificial diets (Støttrup & Mcevoy 2008). Since the amount of microalgae is low in natural seawaters, their cultivation is getting more crucial and required in hatcheries to optimize bivalve feeding process. Moreover, water treatment removes natural

microorganisms including phytoplankton, hence a supply of selected species of microalgae is needed (Helm et al 2006). Several criteria should be taken in consideration, like size and shape of the cells while isolating and culturing microalgae for bivalve feeding purposes (Robert & Trintignac 1997). For instance, the occurrence of a thick wall or theca in some species could lead to some hardness to digest because of the lack of the appropriate enzymes (Muller-Feuga et al 2007). In the hatchery, environmental factors exert an impact on growth productivity of each individual algae species including temperature, salinity, pH and light (Lavens & Sorgeloos 1996; Helm et al 2006; Støttrup & Mcevoy 2008). Therefore, culture conditions have a substantial effect on phytoplankton growth rate and biomass production.

Since many years, several strains were successfully identified and used in bivalve's feeding programs (Helm et al 2006; Muller-Feuga et al 2007). The isolation of new strains with the required size, shape and nutritional value criteria is a crucial step to improve and diversify the local arsenal of strains currently in use (Ouagajjou et al 2018). Also, the use of local strains is highly recommended as they are more adapted to the environmental conditions and have less ecological impact on the environment (Hemaiswarya et al 2011; Ratha et al 2012).

Very few studies were developed along the Moroccan coasts concerning the isolation of local strains that could potentially be used in nutrition programs inside shellfish hatcheries (Ouagajjou et al 2018). To date, biochemical composition in terms of lipids classes, fatty acids, sugars, proteins, vitamins and pigments of the local strains have not been examined to assess how composition relates to differences in the nutritional value of the species. Consequently, this study has been carried out in order to isolate more local microalgae along Moroccan coasts and to evaluate their nutritional value matched to the nutritional requirements in aquaculture.

Material and Method

Sampling for isolation of microalgae. Between February 2015 and September 2017, water samples were collected at the surface level along the Moroccan coastline at several sites including lagoons and open environments (Figure 1). Two methods of sampling phytoplankton were used during this study. The first one was based on sampling by bottle samplers. The second method was based on sampling by net (20 µm mesh size, 25 cm opening diameter and 2 m length).

Microalgae culturing. The experimental set-up for enhancing growth of the total phytoplankton sampled involves cultivation of each sample in 1 mL L^{-1} of culture medium f/2 (Guillard & Ryther 1962; Guillard 1975). For plankton net seawater samples, a preliminary dilution at 1/100 was carried out before adding f/2 medium. All samples were kept in an appropriate incubator EYELA brand, modèle LOW TEMP LTI-1001 SD, under controlled conditions involving temperature 20±0.6°C, artificial lighting intensity 10 µmol m^{-2} s^{-1}. The photoperiod was 16:8 h (light:dark). During the isolation we used f/2 culture medium.

Isolation techniques. A preliminary differential filtration of seawater samples was handled using sieves of different mesh sizes. This step was carried out to target microalgae according to their size. Three methods have been used for the isolation of microalgae cells. The dilution method was based on the inoculation of ten test tubes (10^{-1} to 10^{-10}) containing f/2 culture medium, protocol described in Andersen & Kawachi (2005). After two to four weeks of incubation under the conditions mentioned above, microscopic examination was performed on aseptically harvested aliquots to ensure a single-strain culture. For the solid media method on Petri dishes containing 1.5% agar were prepared also using the f/2 culture medium, protocol described in Andersen & Kawachi (2005). After two to four weeks of incubation, aliquots of colonies were microscopically examined then aseptically transferred to liquid culture media. Isolation of microalgae was also performed by capillary pipetting (single cell), as described by Hoshaw & Rosowski (1973). After three to five days of incubation in the multiwell boxs, cultures were examined under inverted microscope to ensure that a single-strain culture

was obtained. For the isolation of diatom species, a solution of metasilicates was added to the culture medium. Isolates were maintained by repeated inoculation every two weeks to preserve young cells with good multiplication potential (Helm et al 2006).

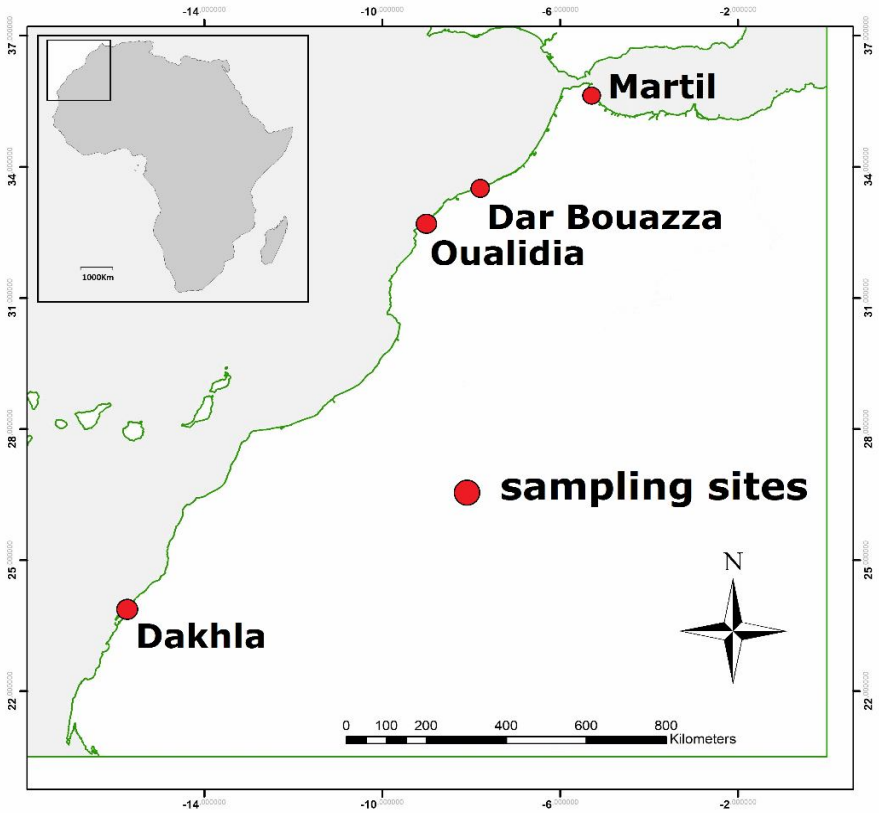

Figure 1. Geographic situation of the sampling sites along Moroccan coast.

Morphological identification. Isolated species were identified under light microscopy using a multitude of determination keys and specialized literature e.g., Delgado & Fortuño Alós (1991), Tomas et al (1993), Tomas (1996) and Al-Kandari et al (2009).

Growth kinetics. To characterize their growth, the isolated strains were grown in batch mode on f/2 medium in 2 L Erlenmeyer flasks (Figure 2). The culture was carried out under specific conditions (temperature 20±0.6°C, salinity 35 g L^{-1}, artificial light intensity 35 µmol m^{-2} s^{-1} and photoperiod 12/12). A permanent bubbling was ensured to homogenize the cultures and atmospheric CO_2 inflow. These conditions were rigorously controlled throughout the experiments to ensure good reproducibility of the culture parameters. Daily growth kinetics were acquired by Malassez hemocytometer, and the following parameters were calculated for each strain, according to Guillard et al (1973).

The specific growth rate (µ, d^{-1}) was calculated according to the equation (1):

$$(1) \quad \mu = \frac{LnXt - LnXt0}{t - t0}$$

Where, Xt and Xt0 are the cell concentrations (cell mL^{-1}) at the end and beginning of the exponential phase, respectively, and t-t0 is the duration of the exponential phase at day.

The division rate (k, d^{-1}) was calculated according to the equation (2):

$$(2) \quad k = \frac{\mu}{Ln\ 2}$$

Doubling time (d, d) was calculated according to the equation (3):

$$(3) \quad d = \frac{Ln\ 2}{\mu}$$

Figure 2. Isolated strains microalgae grown in batch mode on f/2 medium in 2 L Erlenmeyer flasks.

Characterization of size. The size data were acquired by image analysis using the Image J v1.46r program during exponential and stationary phases. The results of the size determination allowed an estimation of the specific biovolumes using the simple geometric shape that best fits the biovolumes according to the standard calculation method used by Olenina et al (2006) and Druart & Rimet (2008).

Determining dry weight. The dry weight of the microalgae cells was determined during exponential phase. A volume of 400 to 800 mL of culture was filtered through a GF/F filter (Whatman GF/F, 47 mm, porosity ranging from 0.7 to 2.7 µm), the cell concentration of this volume was calculated. After filtration, the retained particles biomass was washed three times with deionized water. The filters were then dried in an oven at 80°C until the filter weight was stabilized. Cellular dry weight at (g $cell^{-1}$) was calculated according to the formula of Zhu & Lee (1997), in equation (4):

$$(4) \quad \text{Dry weight of cells} = \frac{\dfrac{[\text{net weight (W1)} - \text{net weight (W0)}]}{\text{filtered volume}}}{\text{cell concentration}}$$

Where, W1 represents the total weight of the filter with microalgae biomass after drying at (g), W0 is the weight of the dry filter alone at (g), filtered volume at (mL) and cell concentration at (cell mL^{-1})

Biochemical analyses. For assess their nutritional value, the strains were grown under the same conditions mentioned above. The biomass was harvested at the end of the exponential phase by centrifugation at 2500 (×g) for 10 min, then freeze-dried and stored at -20°C. Total lipid extraction and dosing was performed according to a modified protocol of Bligh & Dyer (1959) as modified by El Arroussi et al (2017). For the determination of total protein, the method for protein extraction was based on that used by Price (1965) with extensive modifications. A fraction of 10 mg is taken from the dry biomass to which 400 µL of Tri-Chloro-acetic Acid (TCA) at 25% fraction is added and incubated for 15 min at 95°C. The sample is then treated with ultrasound waves at 40 kHz for 15 min. The lysate is placed in ice for 30 min then centrifuged at 35000 (×g) for 20 min at 4°C. The supernatant is then removed, and the pellet was rinsed with TCA (10%) and centrifuged at 35000 (×g) for 2 min at 4°C to remove the supernatant. The third wash is carried out with TCA (5%) followed by 20 min centrifugation at 35000 (×g) for 2 min at 4°C. The pellet obtained is the pure protein extract to which 1.5 mL of ultrapure water has been added. The assay was carried out according to the Bradford method. For the fatty acid (FA) analysis, the fatty acid methyl esters profile was

determined after basic transesterification according to the protocol detailed in (El Arroussi et al 2017).

Statistical analysis. The results were calculated as mean±standard deviation (SD) from three replicates. A one-way analysis of variance (ANOVA) of the results of the growth rates, dry weight and biochemical analysis of each strain, also of the size between the stationary and logarithmic growth phases was performed with a 95% confidence level (α = 0.05). Where significant differences were obtained, a pos-hoc Tukey's test was used. All statistical tests were analyzed using Rcmdr interface (Fox & Bouchet-Valat 2016) implemented in R package version 3.6.2.

Results

Isolation and identification of microalgae. Among 13 isolated native microalgae strains, six (Figure 3) were selected for this study with regard to their growth performance, persistence and adequate size suitable for nutritional purposes in aquaculture. The list of selected strains and their origins are described in Table 1.

Table 1

List of the local isolated strains of microalgae along Moroccan coast

Strain	*Code*	*Isolation date*	*Origin*
Thalassiosira sp. 1	Tha1	Mar. 2015	Martil (35º37'53''N, 5º15'5''E)
Thalassiosira sp. 2	Tha2	Sept. 2017	Dar Bouazza (33º31'49''N, 7º50'3''E)
Chaetoceros sp.	Cha	Sept. 2017	Dar Bouazza (33º31'49''N, 7º50'3''E)
Tetraselmis sp. 1	Tet1	Oct. 2015	Dakhla (23º49'54''N, 15º51'59''E)
Tetraselmis sp. 2	Tet2	Oct. 2015	Dakhla (23º49'54''N, 15º51'59''E)
Nannochloropsis sp.	Nan	Jun. 2016	Oualidia (32º45'10''N, 9º1'16''E)

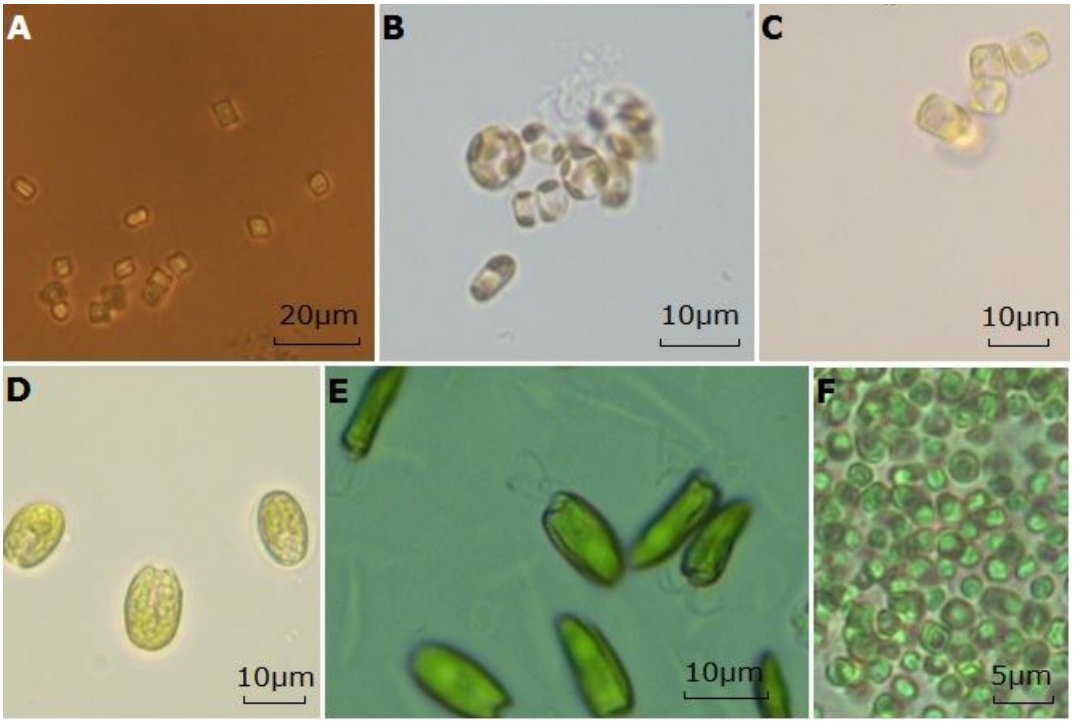

Figure 3. Optical microscope photos of isolated microalgae at different magnifications. (A) *Chaetoceros* sp.; (B) *Thalassiosira* sp. 1; (C) *Thalassiosira* sp. 2; (D) *Tetraselmis* sp. 1; (E) *Tetraselmis* sp. 2, and (F) *Nannochloropsis* sp.

Growth kinetics. The culture results show the classical shape of growth curve with differences in terms of the extent of growth phases within local strains (Figures 4, 5 and 6). *Chaetoceros* sp. (Cha) showed the shortest lag phase (only one day), the stationary

phase has occurred between the fifth and the tenth day with maximum cell concentration 6.7 × 10^6 cell mL^{-1}. The two strains of the genus *Thalassiosira*, *Thalassiosira* sp. 1 (Tha1) and *Thalassiosira* sp. 2 (Tha2) were similar in terms of latency phase, but the strain Tha1 showed a longer exponential phase, their maximum concentrations were (6.7 × 10^6 and 2.9 × 10^6 cell mL^{-1} respectively). As regards to flagellate strains, *Tetraselmis* sp. 1 (Tet1) and *Tetraselmis* sp. 2 (Tet2), Tet1 strain showed a long lag phase (six days), both shown an exponential phase between eight and nine days, with a maximum cell concentration of 2.7 × 10^6 cell mL^{-1} for both. For *Nannochloropsis* sp. (Nan) strain, the growth curve has an almost linear pattern that does not allow a clear distinction between all growth phases. The growth has stopped at the 23rd day of culture with a maximum cell density (148 × 10^6 cell mL^{-1}).

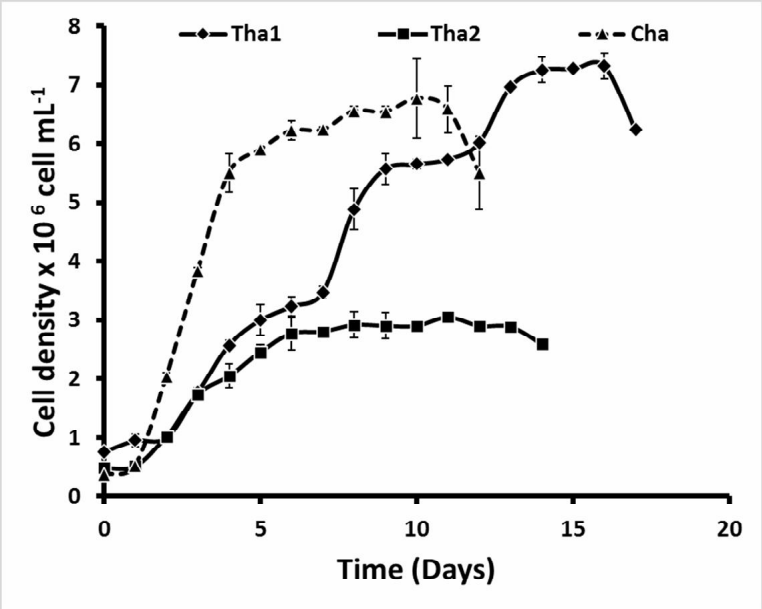

Figure 4. Growth curves of isolated diatoms strains: *Chaetoceros* sp., *Thalassiosira* sp. 1 and *Thalassiosira* sp. 2 (batch culture in f/2 media, temperature (20±0.6°C), salinity 35 g L^{-1}, illumination 35 µmol m^{-2} s^{-1}, and photoperiod 12/12).

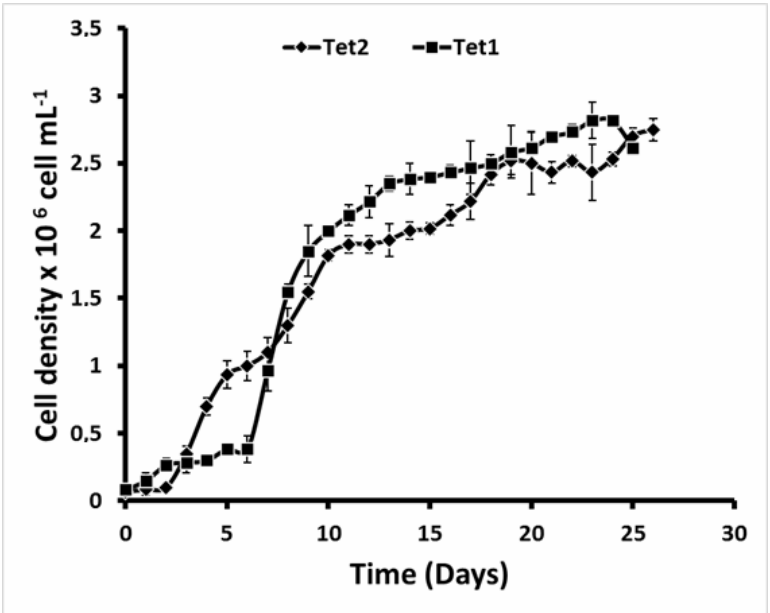

Figure 5. Growth curves of isolated flagellate strains: *Tetraselmis* sp. 1 and *Tetraselmis* sp. 2 (batch culture in f/2 media, temperature (20±0.6°C), salinity 35 g L^{-1}, illumination 35 µmol m^{-2} s^{-1}, and photoperiod 12/12).

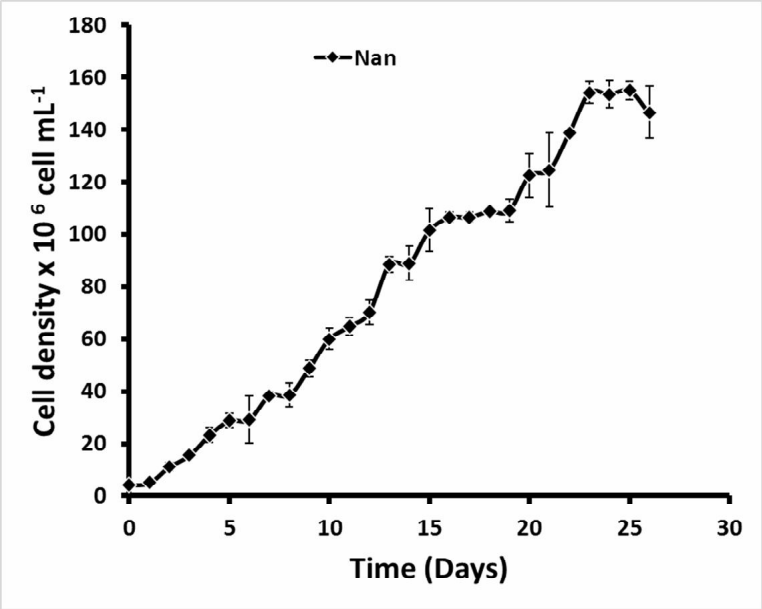

Figure 6. Growth curve of isolated strain *Nannochloropsis* sp. (batch culture in f/2 media, temperature (20±0.6°C), salinity 35 g L^{-1}, illumination 35 µmol m^{-2} s^{-1}, and photoperiod 12/12).

Growth parameters. The Cha strain has significantly the highest growth rate (µ = 0.80 d^{-1}), while a significantly lower growth rate was observed for the two strains Tha1 and Nan (µ = 0.21 d^{-1} and µ = 0.20 d^{-1} respectively) (Table 2).

Table 2

Growth pattern of isolated strains of microalgae (batch culture in f/2 media, temperature 20±0.6°C, salinity 35 g L^{-1}, illumination 35 µmol m^{-2} s^{-1}, and photoperiod 12/12)

Strain	*Max. density (10^6 × Cell Ml^{-1})*	*µ (d^{-1})*	*k (d^{-1})*	*d (d)*
Tha1	6.7±0.63	0.21±0.01	0.30±0.02	3.35±0.23
Tha2	2.9±0.17	0.32±0.05	0.46±0.07	2.19±0.32
Cha	6.7±0.08	0.80±0.00	1.15±0.01	0.87±0.008
Tet1	2.7±0.28	0.42±0.07	0.61±0.10	1.67±0.26
Tet2	2.7±0.06	0.38±0.04	0.55±0.05	1.84±0.18
Nan	148±10	0.20±0.02	0.28±0.02	3.54±0.28)

Abbreviations: µ = growth rate; k = division rate; d = doubling time.

Cells geometric assessment. For size variations according to growth phases (Table 3), Tha2 (N = 600) showed the highest change regarding its geometric properties when it passes from the logarithmic to stationary phase followed by Cha (N = 1278) and Tha1 (N = 1200) strains respectively (t = 14.61 and t = 11.80, p < 0.001). Whereas, the flagellate of the genus *Tetraselmis* cells have evidently increased their strengths (N = 2058) while passing from phase into another (t = -28.39 p < 0.001). The Tet2 cells (N = 1350) have shown a highest increase of their biovolume when they pass from the logarithmic growth onto stationary phase followed by Tet1 (t = -24.36 and t = -16.84 at p < 0.001). Even though the Nan strains have kept their cell properties and showed no difference between the two growth phases (t = -0.73, p < 0.001). Besides the morphological assessment, biovolume and dry cell weight promoted the distinguishing between all strains isolated herein. Henceforth, the biovolume was used to statistically screen the significant difference between two species belonging to the same genera. The analysis of variance for cells that belong Tetraselmis genera has shown a significant difference between the two species Tet1 and Tet2 (F = 10.33, p-value = 0.0013). Moreover, the analysis of variance for cells that belong to *Thalassiosira* genera has shown a highest difference between the two species Tha1 and Tha2 (F = 3805.6, p-value < 2.2e-16).

Table 3

Calculated average cell size, bio-volume and dry weight of the six isolated strains of microalgae

Strain	*Height (µm)*		*Diameter (µm)*		*Bio-volume ($µm^3$)*		*Test t*	*Dry weight ($pg\ cell^{-1}$)*
	H ex	H st	D ex	D st	Bv ex	Bv st	Bv ex/Bv st	
Tha1	7.41±1.28	6.45±1.33	3.67±0.73	3.52±0.66	78.36±1.03	62.9±1.1	11.80***	37.08
Tha2	8.99±0.67	8.03±0.79	5.65±0.62	5.03±0.64	225.56±8.01	159.56±2.13	22.95***	146.18
Cha	6.35±1.4	5.41±1.44	3.8±0.94	3.45±0.94	91.9±4.63	64.54±6.36	14.61***	16.88
Tet1	11.77±1.19	12.83±1.35	7.53±1.07	8.46±1.21	349.37±24.4	480.53±4.28	-16.84***	145.12
Tet2	11.78±1.15	12.72±1.32	7.42±0.79	8±0.87	339.77±8.25	426.72±1.5	-24.36***	190.12
Nan	-	-	2.58±0.24	2.57±0.25	8.95±0.09	8.87±0.17	0.73ns	1.82

Abbreviations: (H) cell mean height, (D) cell mean diameter, (Bv) cell mean bio-volume, (ex) exponential phase, (st) stationary phase, *** significance at $p < 0.001$, and (ns) not significant at $p < 0.001$.

Biochemical analyses. As regards to lipid content (Table 4), Tha1 and Tet1 strains have revealed the highest overall content respectively 57.2% and 51.8% (of dry matter) followed by Tet2, Nan, Tha2, and Cha (35.7%, 34.8%, 31.6% and 26.8% respectively). Protein content (Table 4) was very high in Tet1 and Tet2 (34.1% and 27.9% respectively), followed by Nan, Cha, Tha1 and Tha2 (23.5%, 21.4%, 15% and 13.9% of dry matter respectively).

Table 4

Dry matter, total lipid, and protein contents for culture of the six isolated strains grown in f/2 medium and harvested in the late logarithmic growth phase

Strain	*Cell density 10^6 (cells mL^{-1})*	*Dry matter (mg mL^{-1})*	*Lipid content (%)*	*Protein content (%)*
Tha1	1.28	0.05±0.01	57.2	15±0.2
Tha2	0.97	0.14±0.03	31.6±6.1	13.9±0.6
Cha	2.32	0.04±0.01	26.8	21.4±2.5
Tet1	2.10	0.31±0.04	51.8±5.3	34.1±0.9
Tet2	2.79	0.52±0.31	35.7±3.1	27.9±0.2
Nan	78.2	0.14±0.04	34.8±1.9	23.5±1.4

The fatty acid composition varied markedly between microalgae strains (Table 5).

Table 5

Fatty acid composition (% of total fatty acid) in dry weight of the six isolated microalgae grown in f/2 medium during late logarithmic growth phase

Fatty acids	*Strain*	*Tha1*	*Tha2*	*Cha*	*Tet1*	*Tet2*	*Nan*
		SFA					
13:0	Tridecylic acid	-	-	-	-	0.92	-
14:0	Myristic acid	8.8	15.59	2.32	6.02	0.3	1.52
15:0	Pentadecanoic acid	-	2.19	2.98	0.65	0.4	0.81
16:0	Palmitic acid	33.5	33.11	23.34	33.63	26.4	30.62
17:0	Margaric acid	-	-	4.08	-	-	-
18:0	Stearic acid	39.39	24.7	29.14	27.68	19.26	19.35
20:0	Arachidic acid	-	-	6.45	-	0.62	-
22:0	Behenic acid	-	-	4.30	-	-	-
24:0	Lignoceric acid	-	-	0.74	-	0.46	-
	ΣSFA	81.69	75.59	73.35	67.98	48.36	52.3
		MUFA					
16:1 (n-7)	Palmitoleic acid	-	13.54	-	2.87	3.83	-
18:1 (n-12)	Petroselinic acid	-	-	-	-	4.74	-
18:1 (n-9)	Oleic acid	11.5	4.2	-	5.32	24.29	-
18:1 (n-7)	Vaccenic acid	-	-	-	1.42	-	-
20:1 (n-9)	Gondoic acid	-	-	-	-	1.49	-
	ΣMUFA	11.5	17.74	-	9.61	34.35	-
		PUFA					
16:3 (n-3)	Hexadécatriénoïc acid (HTA)	-	-	-	-	-	13.51
16:4 (n-3)	Hexadecatetraénoïc acid (HDTA)	-	-	-	2.52	-	-
18:2 (n-6)	Linoleic acid (LA)	-	-	19.19	-	-	-
18:3 (n-3)	α-Linolenic acid (ALA)	-	6.67	7.46	14.74	-	34.19
18:4 (n-3)	Stearidonic acid (SDA)	-	-	-	2.25	-	-
20:4 (n-6)	Arachidonic acid (AA)	-	-	-	-	1.82	-
20:4 (n-3)	Eicosatetraeoïc acid (ETA)	-	-	-	-	2.94	-
20:5 (n-3)	Eicosapentaenoic acid (EPA)	6.82	-	-	2.9	12.54	-
	ΣPUFA	6.82	6.67	26.65	22.41	17.3	47.7

Abbreviations: SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids.

For the fatty acid (FA) profile of Tha1, the saturated fatty acids (SFA) occupies almost 81.7% (of total FAs). The mono-unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in Tha1 represents respectively only 11.5% oleic acid and 6.8% eicosapentaenoic acid (EPA). The cells of Tha2 have a composition of the (SFA) up to 75% (of total FAs). In concerns to MUFAs, are only represented by palmitoleic acid (13.5%) and oleic acid (4.2%) (of total FAs). Also, the α-linolenic acid, is the only representative content of the PUFAs for cells of Tha2 (6.7%) (of total FAs). For FAs profile of the diatom Cha strain, the SFAs occupies almost 73.3% (of total FAs). The PUFAs represent 26.6%. Are represented only by the linoleic acid (LA) and α-linolenic acid (ALA) made up 19.2% and 7.5%, respectively. Concerning strains of *Tetraselmis* genus, Tet1 and Tet2, The SFAs and MUFAs of Tet1 strain occupies almost 68% and 9.6% (of total FAs) respectively. In this strain, the PUFAs represent 22.4% (of total FAs), of which the α-Linolenic acid (ALA) and eicosapentaenoic acid (EPA) made up 14.7% and 2.9%, respectively. In the strain Tet2, the SFAs occupy 48.4% (of total FAs). In concerns to MUFAs represent 34.3% (of total FAs). The PUFAs represent 17.3% (of total FAs) that are mainly represented by (EPA)12.5% (of total FAs). Finally, in the strain Nan The SFAs occupy almost 52.3% (of total FAs). In terms of PUFAs composition that accounted for up to 47.7% (of total FAs), are mainly represented by (ALA) and hexadécatriénoïc acid (34.2% and 13.5% respectively).

Discussion. In this study, six microalgae strains were isolated from the Moroccan coastline, they were selected for their high potential-value for replacing the exogenous commercial strains. The choice of local microalgae was first based on ecological preservation issue in order to prevent potential environmental impact of the introduction of exogenous microalgae (Duong et al 2015).

Moreover, before promoting the professional and scientific use of the isolated strains, it is necessary to mention that the cultures obtained herein are not axenic, so they still probably contain a bacterial procession, which is considered somehow desirable. Indeed, the association of bacteria and microalgae often provide enhanced growth (Amin et al 2012). They can stimulate the growth of algae in a variety of ways such as secretion of growth enhancers (Sureshkumar et al 2014).

Growth kinetics. For the isolated species, the lag growth phase was short, especially for diatoms which can be explained by their good acclimation to the experimental environment (Wood et al 2005) and/or to an optimal concentration of the inoculum used to start the culture series (Suantika et al 2009). The time spent before reaching the stationary phase is an important criterion not only in aquaculture production but also in all fields of microalgae's valorization. In this context, the diatoms Cha and Tha1 have shown shortest period to reach the maximum cell density.

Growth pattern. Diatoms generally showed growth performance likely similar comparing to several previous studies on diatoms under different conditions (Suantika et al 2009; Duong 2016). The Cha strain exhibited the highest specific growth rate (μ = 0.80 d^{-1}) which is quiet similar to that obtained for the commercial strain of *Chaetoceros calcitrans* ((Paulsen) H. Takano, 1968) in conditions similar to current study (Shei et al 2008). However, species belonging to this genus have demonstrated some difference in terms of growth rates that varies from 0.14 d^{-1} for *Chaetoceros gracilis* (Pantocsek, 1892) (Shei et al 2008) to 2.35 d^{-1} for *Chaetoceros sp*. 'minus' (native to Argenton-France) in a culture enriched with 3% CO_2 (Robert et al 2004). The specific growth rates obtained for the two strains Tha1 (μ = 0.21 d^{-1}) and Tha2 (μ = 0.32 d^{-1}) are lower compared to growth rate of some species belonging to genus *Thalassiosira* (Robert et al 2004; Duong et al 2015). The growth performances of Tet1 (μ = 0.42 d^{-1}) and Tet2 (μ = 0.38 d^{-1}) strains are lower comparing to the commercial strain *Tetraselmis suecica* ((Kylin) Butcher 1959) (μ = 1.20 d^{-1}) under the same culture condition. Regarding growth performance, the *Tetraselmis* genus strains isolated herein are more likely like the performance of strain published in several studies (Lim et al 2012; Duong et al 2015; Ouagajjou et al 2018). Whereas, the growth pattern is not a basic criterion to identify

strains taxonomy while for instance the commercial strain have revealed unlike performances under different conditions (Robert & His 1985; Robert et al 2004; Huerlimann et al 2010; Lee et al 2011; Khatoon et al 2018). For the Nan strain, the specific growth rate ($\mu = 0.20\ d^{-1}$) remains slightly higher than that of *Nannochloropsis oculata* ((Droop) D. J. Hibberd, 1981) ($\mu = 0.13\ d^{-1}$) (Converti et al 2009), but very low regarding growth performance of other strains of the *Nannochloropsis* genera (Huerlimann et al 2010; Bae & Hur 2011).

Geometric assessment. To serve as trophic source for bivalve in captivity, the isolated microalgae must be appropriate from eco-physiological point of view in terms of nutritional value and shape size. The average size of the selected strains isolated herein ranges from 2.57±0.25 to 12.83±1.35 µm, which represents a wide range of sizes to feed a variety of bivalves at different stages of development. For larvae stage, their feeding shortly begins after shell and velum development (Gosling 2015). Two-day old larvae, with an average length of 100 µm, they can actively ingest food particles as small as 0.5 µm in diameter (Gallager 1988). The upper limit ingested by the larvae depends on the opening of the mouth and oesophagus apparatus of the bivalves. For instance, bivalve larvae with an average length of less than 130 µm are unable to ingest cells larger than 10 µm (Riisgard et al 1980; Fritz et al 1984; Baldwin & Newell 1991). The sizes of the four strains Tha1, Tha2, Cha, and Nan are therefore adequate for the nutrition from early larvae stage, whereas the Tet1 and Tet2 strains cannot be introduced into the diet until larvae exceed 130 µm in size, including the post-larval stages.

Biochemical profile. The different classes of microalgae differ significantly as regards their biochemical composition that highly influences their nutritional value. Both local strains of *Thalassiosira* genera have shown considerable fats richness (57.2% and 31.6% for Tha1 and Tha2 respectively) comparing to other species of the same genus such as *Thalassiosira pseudonana* (Hasle & Heimdal, 1970) that fats content ranges from 19% to 24% (Brown 1991; Helm et al 2006). Protein content was relatively low (15% and 13.9% for Tha1 and Tha2 respectively) comparing to *T. pseudonana* species with a protein content of 34% (Brown 1991). The same proven result was proven for the isolated Cha strain in terms of lipid and protein contents (27% and 21% respectively). These important contents are much higher compared to other species of the genus *Chaetoceros* which vary from 7.2 to 19% for lipids (Brown 1991; Lavens & Sorgeloos 1996; Helm et al 2006; Ohse et al 2015) and from 12 to 34% for protein (Brown 1991).

Both strains of the genus *Tetraselmis* showed considerable lipid richness (52% and 36% for Tet1 and Tet2 respectively) that matches considerably with previous results on local unspecified strain of the genus *Tetraselmis* (El Arroussi et al 2017). Regarding protein content, the local strains of the genus *Tetraselmis* showed also considerable proportions of protein (34% and 28% for Tet1 and Tet2 respectively) which are quite similar for *T. suecica* (31%) (Brown 1991). The Nan strain has revealed a much higher lipid content (35%) than other species of this genus which ranges for *N. oculta* from 18% (Brown 1991) to 22-24% (Converti et al 2009) depending on culture conditions. Protein fraction of this strain remains very low (23.5%) comparing to that assessed in *N. oculata* (35%) (Brown 1991).

For fatty acid profiles, all the isolated strains have revealed high content of SFA that are mainly represented by palmitic acid and stearic acid (ranging from 23 to 33% and from 19 to 39% of total FAs respectively). For Nan strain, the high percentage accounted of the palmitic acid, was consistent with many studies carried on this species (Jiménez-Valera et al 2016; Aussant et al 2018; Chen et al 2020). *Tetraselmis* strains genus have shown also high amount of palmitic acid which is generally similar and reliable regarding the results proven in several studies on species that belongs to the same genus (Huerlimann et al 2010; Jiménez-Valera et al 2016). The *Thalassiosira* and *Chaetoceros* strains genera have revealed also the same scenario of high level of palmitic acid which is consistent with studies handled on species belonging to these genera (Viso & Marty 1993; Mitani et al 2017). Concerning MUFA, oleic and palmitoleic acids are the major representative component of this category in all isolated strains. Tet2, Tha1 and

Tet1 strains have accounted systematically an important amount of oleic acids respectively which fits systematically within the ranging levels fixed base on growth phase and medium culture (Huerlimann et al 2010). Conversely, palmitoleic acid accounted for more than 13% of total FAs in the strain Tha2 which is consistent with previous study carried on *T. pseudonana* (Volkman et al 1989). In terms of PUFA, the high level of α-Linolenic acid (ALA) and hexadecatrienoic acid (HTA) respectively made up to 47% of total FAs in Nan. This composition corresponds to other published work on local similar species (El Arroussi et al 2017) but differ too much with other studies (Huerlimann et al 2010). The genus *Tetraselmis* strains had completely different composition of PUFA. Tet1 cells contain an important amount of ALA which is more or less similar and consistent with several publish work (Volkman et al 1989; Huerlimann et al 2010; Jiménez-Valera et al 2016). On the other hand, the eicosapentaenoic acid (EPA) was accounted in Tet2 as the major component of PUFA (more than 12% of total FAs) which is very high and not consistent at all with lower level (less than 6%) revealed in many published studies (Volkman et al 1989; Patil et al 2007; Huerlimann et al 2010). The linoleic acid (LA) was the third more important FA component of total FAs in Cha. This PUFA was very high and differ from other species of the *Chaetoceros* genus (Volkman et al 1989; El Arroussi et al 2017; Mitani et al 2017). The two strains Tha1 and Tha2 have only shown one component of PUFA for each at a low amount not exceeding 7% (EPA and ALA respectively). This amount fluctuates for EPA depending on species and culture conditions and ranges from 2 to 19% (Volkman et al 1989; Ohse et al 2015), and for ALA it is 6.69% in the species *Thalassiosira fluviatilis* (Hustedt, 1926).

Conclusions. The growth and Biochemical profile of the microalgae strains that we isolated differed among species. Generally, the strains have showed verified performance but it is well known that many factors can strongly influence the growth and composition of microalgae, it is therefore necessary to seek to optimize growing conditions for the isolated strains to increase growth yield. Thus, the microalgae isolated and partially characterized in this work, can be used alone or as part of a mixed diet to feed bivalves larvae, juvinil and adults.

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