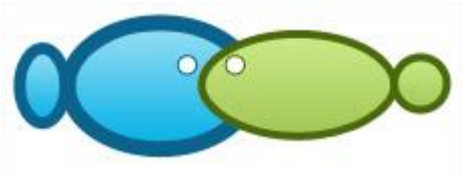

Effects of different diets based on newly isolated microalgae from the Moroccan coastline on the growth and survival of *Crassostrea gigas* spat

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Abstract. Five local strains of microalgae were isolated from the Moroccan coast, and their dietary value in various diets composition (31 diets) was evaluated and compared on oyster *Crassostrea gigas* spat. The results of the feeding tests showed that the composition of the diet greatly influenced the growth and survival of spats. In the single-strain microalgae diets, *Tetraselmis* sp1 and *Tetraselmis* sp2 supported the highest growth rates, while the *Nannochloropsis* sp. strain resulted in the lowest growths. With regard to survival rate, the highest value was obtained by the *Chaetoceros* sp. strain. For the mixed strains algal diets, two diets showed the highest growth rates. The first one comprised *Chaetoceros* sp. and *Tetraselmis* sp2 in a two-strain diet and the same strains plus *Tetraselmis* sp1 for the second. The majority of the diets containing *Thalassiosira* sp1 and/or *Nannochloropsis* sp. resulted in slight increase of size. The mixed diets that showed the highest survival rates contained in their composition either *Thalassiosira* sp1 or *Chaetoceros* sp. with *Tetraselmis* sp1 or *Tetraselmis* sp2, while the lowest survival rates were generally associated with the *Nannochloropsis* sp. strain in the composition of the diets.
Key Words: biochemical composition, bivalve culture, shellfish farming, shellfish hatcheries, shellfish nutrition.

Introduction. Bivalve molluscs make an important contribution to aquaculture production (FAO 2020), of which bivalves make up a large proportion. The main bivalve organisms produced by aquaculture are oysters, clams, mussels, and Pectinidae (Richmond 2007). In Morocco, oyster culture alone accounts for 61% of national marine aquaculture production (ANDA 2016). Aquaculture production of bivalves relies heavily on the cultivation of appropriate microalgal species to provide essential nutrients for growth and survival. The culture of microalgal species is significant to marine bivalve hatcheries generally, as they are the only suitable food source (Støttrup & Mcevoy 2008), with new solutions such as the use of yeast, bacteria, microparticles, sludge, paste, or dried and frozen microalgae not being sufficiently advanced to date to offer an alternative to live microalgae (Robert & Trintignac 1997; Richmond 2007).

For a microalga to be considered as a good source of nutrition for bivalves in a hatchery, in addition to their easy and sufficient producibility, their compatible size, their digestibility which must be easy (Robert & Trintignac 1997), it must be of good feed value (Muller-Feuga et al 2007). In all these aspects, bivalves' microalgal requirements differ for the different taxonomic groups of bivalves (Aranda-Burgos et al 2014; Yang et al 2016), and variations in food preferences between broodstock, larvae and post-larval rearing have also been confirmed (Støttrup & Mcevoy 2008).

Bivalves, unlike fish and crustaceans, are fed directly from microalgae cells from a young age. Therefore, the successful development of bivalves in culture is closely linked to the microalgae's quantity and quality produced for their feed (Muller-Feuga et al 2007). High quality microalgae are particularly important for feeding early-stage bivalves

(Webb 1983; Robert & Trintignac 1997; Knauer & Southgate 1999) and broodstock conditioning (Richmond 2007; Støttrup & Mcevoy 2008). In aquatic ecosystems, microalgae occupy the lower end of the food chain. They are also the main source of polyunsaturated fatty acids, such as eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid (Brown et al 1997). The polyunsaturated fatty acid profiles of microalgae have long been considered an indicator of high nutritional quality, promoting good growth and high survival in hatchery and nursery production of bivalves (Ronquillo et al 2012).

The microalgae used differ in their biochemical composition. There is no single microalgae species that can satisfy all the nutritional requirements of developing bivalve larvae and juveniles (Ronquillo et al 2012). Combined and complementary feeding is essential to ensure the success and reduce the resources of running a bivalve hatchery and nursery. A mixed diet of two or more algal species fed to bivalves has been shown to provide a better balance of nutrients (Brown et al 1998). Also, in bivalve larvae, underfeeding can result in poor growth and survival rates. Overfeeding can cause increased hatchery costs and may result in poor water quality (Doroudi et al 1999). Therefore, determining the appropriate diet compositions and combinations becomes very important.

In this study, several microalgae that were found to be suitable as food for bivalve molluscs in different developmental stages were isolated from natural waters of the Moroccan coast (Elyakoubi et al 2020). The main objective of the study was to evaluate the nutritional potential in aquaculture of five strains of the isolated microalgae, in single and mixed diets, by testing all possible combinations in relation to their biochemical compositions, in a feeding study of Pacific oyster (*Crassostrea gigas*) spat, in order to determine the best performing combinations for spat growth and survival. The results can omprove the selection of optimal diets for hatchery production of oyster spat and other high-value bivalve species.

Material and Method

Microalgal strains. The five microalgal strains tested in this study (Table 1) were isolated from four sites along the Moroccan coast. In order to produce the biomass necessary for the feeding of oyster spat, the microalgae cultures were initiated in 250-500 mL Erlenmeyer flasks and then transferred to 2L Erlenmeyer flasks when they reached the exponential growth phase. These cultures were carried out at a temperature of 20°C±0.6, under continuous artificial lighting (24 hours) diffused by fluorescent lamps (35 µmol m-2 s-1). Bubbling was provided to homogenise the cultures and to allow for a supply of CO_2. After six to eight days, the cultures were transferred to 5L bottles under the same conditions. The microalgae used to feed the spat came from the beginning of the stationary phase of the cultures.

These strains were selected following cytomorphological and biochemical characterisation and a study of growth kinetics. Two diatoms, *Thalassiosira* sp1 (Tha) and *Chaetoceros* sp. (Cha), two Chlorodendrophyceae of the genus *Tetraselmis* (Tet1 and Tet2), and one Eustigmatophyceae of the genus *Nannochloropsis* (Nan).

The cell sizes of the selected strains range from 2.57±0.25µm to 12.83±1.35µm. All these selected strains do not have spines, a criterion that is important since their ingestion is easier for oyster spat.

Table 1

Characteristics of the microalgae strains used to feed the spat (Elyakoubi et al 2020)

Strain		*Thalassiosira* sp. 1	*Chaetoceros* sp.	*Tetraselmis* sp. 1	*Tetraselmis* sp. 2	*Nannochloropsis* sp.
Code		Tha1	Cha	Tet1	Tet2	Nan
Origin		Martil (35º37'53''N, 5º15'5''E)	Dar Bouazza (33º31'49''N, 7º50'3''E)	Dakhla (23º49'54''N, 15º51'59''E)	Dakhla (23º49'54''N, 15º51'59''E)	Oualidia (32º45'10''N, 9º1'16''E)
Taille (µm)		7.41±1.28	6.35±1.4	11.77±1.19	11.78±1.15	2.58±0.24
Growth rate (division day^{-1})		0.21±0.01	0.80±0.00	0.42±0.07	0.38±0.04	0.20±0.02
Dry weight (pg cell^{-1})		37.08	16.88	145.12	190.12	1.82
Protein content (% of Dry matter)		15±0,2	21,4±2,5	34,1±0,9	27,9±0,2	23,5±1,4
Lipid content (% of Dry matter)		57,2	26,8	51,8±5,3	35,7±3,1	34,8±1,9
Fatty acids (% of total fatty acid)						
13:0	Tridecylic acid	-	-	-	0.92	-
14:0	Myristic acid	8.8	2.32	6.02	0.3	1.52
15:0	Pentadecanoic acid	-	2.98	0.65	0.4	0.81
16:0	Palmitic acid	33.5	23.34	33.63	26.4	30.62
17:0	Margaric acid	-	4.08	-	-	-
18:0	Stearic acid	39.39	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	73.35	67.98	48.36	52.3
16:1 (n-7)	Palmitoleic acid	-	-	2.87	3.83	-
18:1 (n-12)	Petroselinic acid	-	-	-	4.74	-
18:1 (n-9)	Oleic acid	11.5	-	5.32	24.29	-
18:1 (n-7)	Vaccenic acid	-	-	1.42	-	-
20:1 (n-9)	Gondoic acid	-	-	-	1.49	-
ΣMUFA		11.5	-	9.61	34.35	-
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)	-	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	26.65	22.41	17.3	47.7

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

Oyster spat. The feeding experiments were carried out on diploid *C. gigas* spat from a single spawning batch. The spat were acclimatized to laboratory conditions for one week and fed a diet consisting of the five isolated microalgal strains. Their average size after acclimatization was 5.91±0.29 mm.

Experimental set-up and rearing conditions of the oyster spat. The spat rearing device consists of a 12L polypropylene plastic tank inside which a cylinder sieve is placed to keep the spat suspended in the water. Aeration is provided by a bubbler. The flow of air injected by this bubbler also prevents the sedimentation of the distributed microalgae. It avoids the spat's grouping so that the oysters do not form clusters as they grow. The seawater used for spat rearing is filtered through a sand filter and treated with UV light. Its temperature is maintained between 18 and 20°C; its salinity is between 34 and 36 g L^{-1}, and its pH between 8 and 8.3. The spat density used is calculated according to the size of the individuals according to Helm et al (2006).

Species composition of diets. A total of 31 diets (Table 2), consisting of combinations of the isolated microalgal strains, were tested. Five diets were single-strain diets; the others were mixed diets, consisting of all five strains in equal proportions.

Table 2

Compositions and species proportions in different diets.

Diets		Compositions and species proportions
Single-strain microalgae diets	*D1*	100% (Cha)
	D2	100% (Tha1)
	D3	100% (Tet1)
	D4	100% (Tet2)
	D5	100% (Nan)
Mixed strains microalgae diets	*D6*	50% (Cha) + 50% (Tha1)
	D7	50% (Cha) + 50% (Tet1)
	D8	50% (Cha) + 50% (Tet2)
	D9	50% (Cha) + 50% (Nan)
	D10	50% (Tha1) + 50% (Tet1)
	D11	50% (Tha1) + 50% (Tet2)
	D12	50% (Tha1) + 50% (Nan)
	D13	50% (Tet1) + 50% (Tet2)
	D14	50% (Tet1) + 50% (Nan)
	D15	50% (Tet2) + 50% (Nan)
	D16	33,3% (Cha) + 33,3% (Tha1) + 33,3% (Tet1)
	D17	33,3% (Cha) + 33,3% (Tha1) + 33,3% (Tet2)
	D18	33,3% (Cha) + 33,3% (Tha1) + 33,3% (Nan)
	D19	33,3% (Cha) + 33,3% (Tet1) + 33,3% (Tet2)
	D20	33,3% (Cha) + 33,3% (Tet1) + 33,3% (Nan)
	D21	33,3% (Cha) + 33,3% (Tet2) + 33,3% (Nan)
	D22	33,3% (Tha1) + 33,3% (Tet1) + 33,3% (Tet2)
	D23	33,3% (Tha1) + 33,3% (Tet1) + 33,3% (Nan)
	D24	33,3% (Tet1) + 33,3% (Tet2) + 33,3% (Nan)
	D25	33,3% (Tha1) + 33,3% (Tet2) + 33,3% (Nan)
	D26	25% (Cha) + 25% (Tha1) + 25% (Tet1) + 25% (Tet2)
	D27	25% (Cha) + 25% (Tha1) + 25% (Tet1) + 25% (Nan)
	D28	25% (Tha1) + 25% (Tet1) + 25% (Tet2) + 25% (Nan)
	D29	25% (Cha) + 25% (Tet1) + 25% (Tet2) + 25% (Nan)
	D30	25% (Cha) + 25% (Tha1) + 25% (Tet2) + 25% (Nan)
	D31	20% (Cha) + 20% (Tha1) + 20% (Tet1) + 20% (Tet2) + 20% (Nan)

Daily feed rations. The feed ration used was 0.4, i.e., 0.4 mg dry weight of algae per 1 mg fresh weight of spat was provided per week (Helm et al 2006). This ration expressed in dry weight of algae demanded was calculated according to the following equation (1):

$$F = \frac{S * R}{7} \quad (1)$$

where F is the dry weight of algae (mg) per day. R is the Ration in dry weight of seaweed (mg) per milligram of fresh weight of spat per week. S, the fresh weight of spat (mg) at the beginning of each week.

The volume of each algae required to ensure this quantity is calculated according to the dry weight of the strain in question and the cell density at harvest, following equation (2):

$$V = \frac{S * R}{7 * P * C} \quad (2)$$

where V is the volume of harvested seaweed required for a daily ration in litres. P is the dry weight of one million algal cells of the requested strain: C, the harvest cell concentration of the considered strain (cells/µl).

Cell densities were determined by hemacytometry (counting cells under a microscope with a MALASSEZE slide). The dry weight of the cells was determined by filtration of 400 to 800 mL of culture at known cell concentration. After filtration, the retained biomass was washed three times with deionised water; the filters were then dried in an oven at 80°C until the filter's weight was stabilised. The dry weight of the cells is calculated using equation (3) (Zhu & Lee 1997):

$$\text{Cell dry weight } \left(\frac{\text{g}}{\text{cell}}\right) = \frac{\frac{\text{Net dry weight of biomass after filtration (g)}}{\text{filtered volume (mL)}}}{\text{Cell concentration } \left(\frac{\text{cell}}{\text{mL}}\right)} \quad (3)$$

Monitoring of spat growth and survival. The feeding trial lasted eight weeks, between May and June 2018 at the central laboratory of the National Institute of Fisheries Research (INRH) in Casablanca. Survival rate was calculated weekly. Spat growth was monitored by weekly measurement of spat shell length.

The instantaneous relative growth rate (k) was calculated from the size increase data measured weekly (Dehnel 1955), using the following equation (4):

$$k = \frac{lnN1 - lnN0}{t} \quad (4)$$

where N1: the length of the shell at time t1; N0: the shell's length at time t2. Time is expressed in weeks.

The survival rate is calculated according to the following formula (5):

$$S = (S1 - S0) * 100 \quad (5)$$

where S1: the number of individuals in the tank at the end of the experiment, S0: the number of individuals in the tank at the beginning of the experiment, and t: the number of weeks of the experiment.

Spat clearance rate per microalgal strain. Spat clearance was monitored according to the strains fed by determining the cell concentration in the single-strain feeding tanks. Concentrations were determined from the first hour of feeding and during each hour for five hours in a row (Ponis et al 2006).

The clearance rates of microalgae by the spat were calculated according to the following equation (6):

$$\text{CR} = \frac{\text{c1} - \text{c2}}{\text{c2}} * 100 \quad (6)$$

where C1: Initial concentration of microalgae in the medium; C2: Final concentration.
Statistical analysis. The difference in growth between the different batches of oysters at the end of the experiment was tested by one-way ANOVA ($p<0.05$). The normality of the data was previously checked by the Shapiro-Wilk test and the homogeneity of variances by the Bartlett test. The Kruskal-Wallis test ($p<0.05$) was used when the ANOVA assumptions were not met. These statistical analyses were performed using R Studio version 1.2.1335 ©.

Results

Single-strain microalgae diets.
Relative instantaneous growth rate. Growth rates showed significant differences among the different single-strain microalgae diets used ($p<0.05$) (Figure 1). The highest growth rate was obtained with the diet based on the Tet2 strain and the lowest with the Nan strain. The rate obtained by the Tet1 strain was also significant. Comparison of the growth rates obtained by the different diets showed no significant difference between those of the Tet1 and Tet2 strains and between those of Tha1 and Cha ($p<0.05$) (Figure 1). However, the growth rates obtained by other two strains were significantly lower compared to those obtained by the two previous strains ($p<0.05$) (Figure 1).

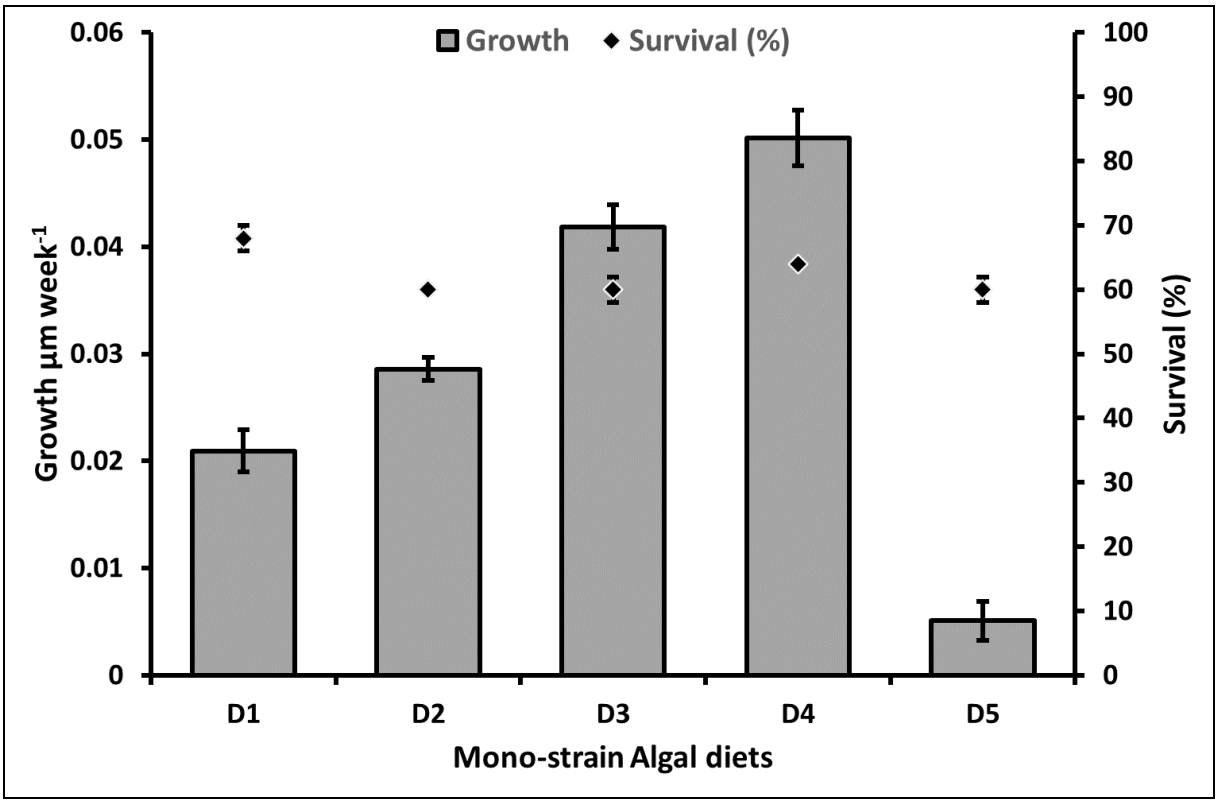

Figure 1. Instantaneous relative growth rate (K), based on the shell size of oyster *Crassostrea gigas* spat, fed with different single-strain microalgae diets of live microalgae with feed ratio of 0.4.

Survival rate. The maximum survival rate of spat fed single-strain diets was obtained with the Cha strain (68%), followed by Tet2 strain (64%). The other strains achieved a survival rate of 60% (Figure 2).

Clearance rates. The clearance rate of microalgae in monospecific tanks shifted between 18±2% (Nan) to 84±5% (Cha after five hours). The clearance rates of Tet1, Tet2, and Tha1 were 71±4%, 67±2% and 61±4%, respectively.

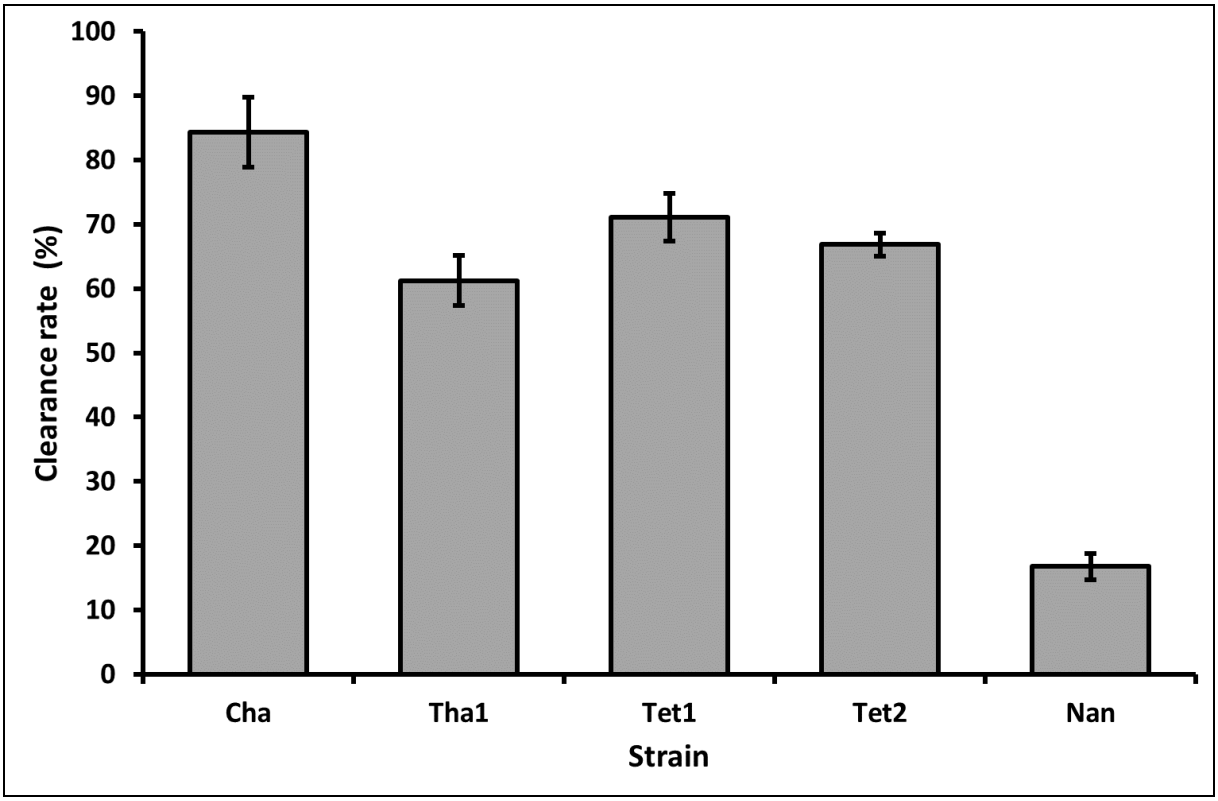

Figure 2. Clearance rates of *Crassostrea gigas* spat fed various single-strain microalgae diets, calculated following monitoring of filtration over five hours in a 12 L tank.

Mixed strains algal diets.

Instantaneous relative growth rate. At the end of the experiment, all spat reared under mixed strains algal diets demonstrated a highest growths in size ($p<0.05$). However, the instantaneous relative growth differed among diets. Diets D8, D13, and D19, showed remarkable performance; they increased the average size from 5.9±0.04 mm to 11.83±0.14 mm, 10.65±0.2 mm and 11.37±0.1 mm, respectively. The D8 diet is composed of the two strains Cha and Tet2 (bispecific), the D13 diet was composed of the two strains Tet1 and Tet2 (bispecific) and D19 diet is trispecific composed of these three strains. The spat fed on the diets D13, D21 and D29 showed moderate growth. These diets contained these three strains, in different specific diets, while, lowest growth wre obtained in the diets consisting of the Tha1 strain or the Nan strain alone or mixed with other strains.

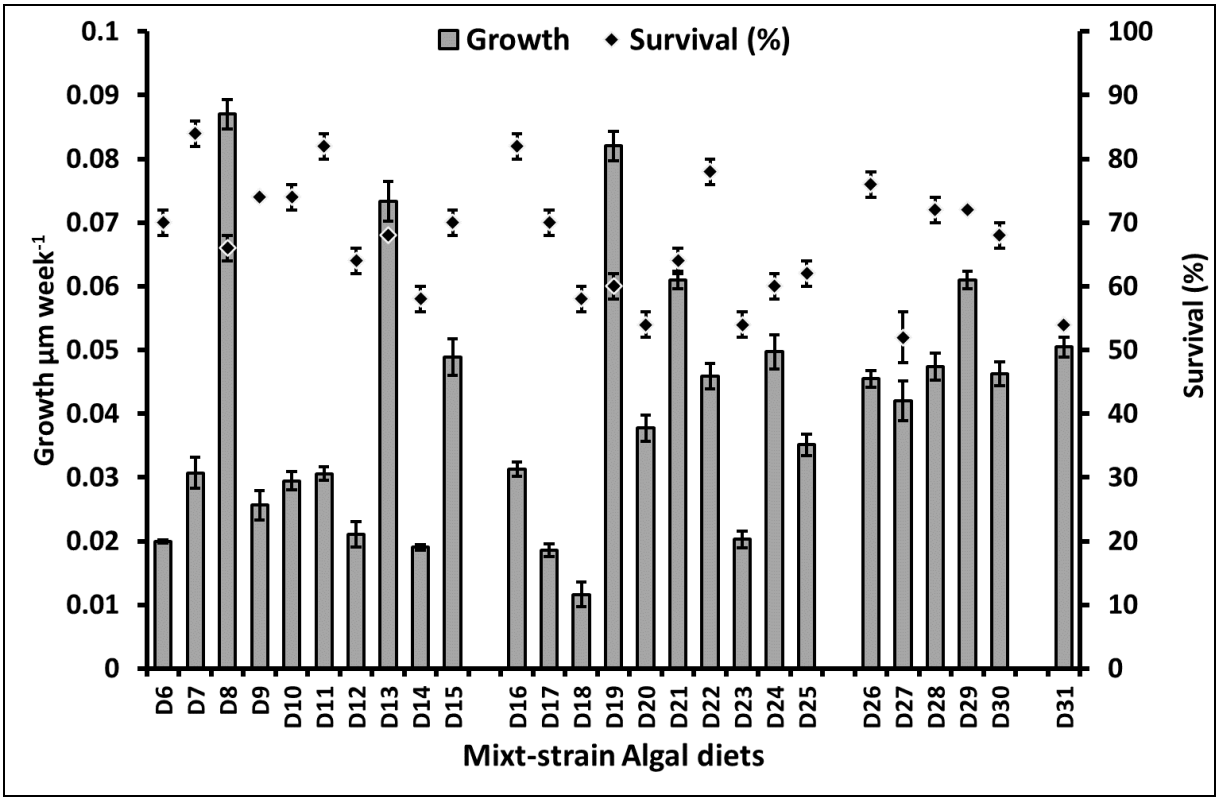

Figure 3. Instantaneous relative growth rate (K), based on spat shell size of oysters *Crassostrea gigas* fed different mixed strains algal diets of live microalgae with feed ratio of 0.4.

Survival rates. In different mixed diets tested, highest survival rates (more than 80%) were obtained in D7, D11 and D16 (Figure 3). In contrast, nine of the other diets, resulted in survival rates compromised between 70% and 80%.

Discussion

Single-strain microalgae diets. Feeding tests were essential to select the optimal diet. All the single-strain diets resulted in more or less appreciable spat growth and survival rates exceeding 60%. All selected strains can be used as aquaculture feed for *C. gigas* spat. However, due to their nutritional composition, this diets resulted in different growth performance. Several species of the genus *Tetraselmis* are frequently used in bivalve hatcheries (Brown et al 1997; Reitan et al 1997; Kawamura et al 1998). Previous studies have shown a performance of *Tetraselmis suecica* on the growth of *Ruditapes decussatus* spat (Albentosa et al 1996; Marquez et al 2019). *Tetraselmis* spp. are microalgae with a good balance of essential amino acids (Walne 1970; Laing & Millican 1986). In our study, the two strains Tet1 and Tet2 tested gave the best growth performance of *C. gigas* spat, also, no significant differences in growth and survival rates were observed between spat fed with these two strains. Furthermore, some studies have shown that variations in the biochemical composition of microalgae of the same genus have remarquable effects on the growth and survival of bivalves (Ponis et al 2006; Marquez et al 2019). Similarly, slight differences in fatty acids such as EPA in two microalgae of the same genus resulted in different shell growths in juveniles of the bivalve *Spondylus limbatus* (Marquez et al 2019).

The influence of the biochemical composition of microalgae on bivalve growth has been widely demonstrated (Brown et al 1997; Robert & Trintignac 1997; Aranda-Burgos et al 2014). Especially, the important nutritional role of essential polyunsaturated fatty acids (Brown 2002; Martínez-Fernández et al 2006; Aranda-Burgos et al 2014; Yang et al 2016), thereby, Tet1 is rich in 18:3 (n-3) α-Linolenic acid (ALA) (14.74%), and also contains EPA (2.9%), while Tet2 is rich in EPA (12.54%) and is the only strain to contain arachidonic acid (1.82%) who is recognised as most important for growth of *Placopecten*

magellanicus post-larvae (Milke et al 2008). On the other hand, ALA is considered the precursor from which some bivalve molluscs synthesise other long-chain polyunsaturated fatty acids like C20 and C22 (De Moreno et al 1976; Waldock & Holland 1984; Chu & Greaves 1991; Rivero-Rodríguez et al 2007). As the low growth of *Ostrea corteziensis* juveniles fed on *Tetraselmis suecica* has been associated with its low PUFA content (Rivero-Rodríguez et al 2007), the same result has been reported for *Ostrea edulis* juveniles fed on *Tetraselmis striata* (Ronquillo et al 2012).

In terms of clearance, a significant difference was observed. The spats filtred more cells from Tet1 compared to Tet2. This difference can be explained by the fact that Tet1 cells tend to aggregate into clusters of several cells which made them readily available to the spats. This trait has also been observed in other species of the genus *Tetraselmis* (Brown et al 1998).

The high performance of the two strains of the genus *Tetraselmis* compared to the other microalgae studied is not only due to their polyunsaturated fatty acid content but also to their protein content (34.1% in Tet1 and 27.9% in Tet2). Knuckey et al (2002) showed that the performance of diets on the growth of *C. gigas* juveniles is mainly correlated to their protein content. Brown et al (1998) have also shown a correlation between the growth rate of oyster spats and the protein and carbohydrate rations consumed when fed single-strain diets. Similarly, Kreeger and Langdon (1993) and Knuckey et al (2002) have shown that the growth of *Mytilus trossulus* mussels juveniles is related to the bioavailability of protein in the diet.

On the other hand, Robert et al (2001) found that a single-strain diet based on *T. suecica* resulted in low growth associated with high mortality in umbonate *C. gigas* larvae compared to mixed diets, an equivalent result was observed in *Pecten maximus* spat (Laing & Psimopoulous 1998). According to Robert and Trintignac (1997), the poor performance of diets composed of strains of the genus *Tetraselmis* is related to the indigestibility of the cells by the bivalves. However, microscopic observation of spat faeces fed single-strain diets based on Tet1 or Tet2 showed only rare intact cells, which confirms their good digestion.

Microalgae of the genus *Thalassiosira* are known to be used as feed in aquaculture. The two species, *Thalassiosira pseudonana*, and *Thalassiosira weissfloggii* are widely used in bivalve hatcheries (Coutteau & Sorgeloos 1992; Borowitzka 1997; Lee 1997; Reitan et al 1997; Kawamura et al 1998).

In this study, spats fed by Tha1 showed only a moderate instantaneous relative growth rate and a lower survival rate compared to the other strains studied. The analysis of the biochemical composition of the species showed that most of its fatty acids are saturated (81.7%) and contain only one polyunsaturated fatty acid (6.82% EPA), this strain is also characterised by a low protein content (15%). The clearance rate for this strain is also lower than the other species studied.

Several studies have looked at the nutritional value of the genus *Thalassiosira* and its performance in shellfish aquaculture. Ronquillo et al (2012) showed that *T. weissfloggii* allowed only a low growth rate in *Ostrea edulis* juveniles due to its low polyunsaturated fatty acid content. For razor clam, *Sinonovacula constricta*, Yang et al (2016) showed that *T. weissfloggii* resulted in low growth rate and high mortality for small spats, however it showed good performance for the large ones, and similar results were obtained with *T. pseudonana* (Yang et al 2016). For the bivalve *Paphies australis* this microalga performed only moderately well in larvae and postlarvae (Mamat & Alfaro 2014).

Spat fed with the Cha strain of the genus *Chaetoceros* showed the lowest instantaneous relative growth rate but with the highest survival rate (68%). The biochemical composition of this species revealed the absence of the essential fatty acids EPA, ARA, and DHA. However, it contained 7.46% ALA, this component can be used for the synthesis of other long chain polyunsaturated fatty acids (De Moreno et al 1976; Waldock & Holland 1984; Chu & Greaves 1991; Rivero-Rodríguez et al 2007). In terms of clearance, this strain showed the highest rate of clearance, but this does not necessarily reflect the importance of this strain. According to Laing and Millican (1986), the spat increases its filtration rate to compensate the low nutritional value of the diet.

Species of this genus are widely used in bivalve hatcheries and nurseries (Borowitzka 1997; Brown et al 1997; Helm et al 2006). The species *Chaetoceros calcitrans*, *Chaetoceros gracilis* and *Chaetoceros muelleri* are the most commonly used. The first species has been considered a complete diet for *O. edulis* spat, allowing good growth (Laing & Millican 1986), it also demonstrated its nutritional efficiency on *P. maximus* spat compared to *T. suecica* (Laing & Psimopoulous 1998) and *C. corteziensis* spat (Rivero-Rodríguez et al 2007).

The diet based on the Nan strain of the genus *Nannochloropsis* showed the poorest performance in terms of growth and survival rate of *C. gigas* spat despite its richness in α-Linolenic acid (34.19%). The clearance rate reached by the spats is also low (16.8% in five hours), which may explain its poor performance. In addition, microscopic examination of the faeces of the spats showed the presence of a large number of intact cells of Nan. Several studies have attributed the poor performance of species of this genus to their fibrous glycoprotein cell wall which makes them poorly digestible by bivalve spats (Lora-Vilchis & Maeda-Martinez 1997; Martínez-Fernández et al 2004; Marquez et al 2019). However, the species *Nannochloropsis occulata* has been frequently used in mollusc hatcheries (Brown 1991; Coutteau & Sorgeloos 1992; Lavens & Sorgeloos 1996; Robert & Trintignac 1997).

None of the strains studied contained docosahexaenoic acid (DHA), furthermore, this polyunsaturated fatty acid is considered important for the growth and survival of bivalves (Soudant et al 1997). Studies have shown that bivalve larvae exhibit DHA in their tissues while being fed microalgae lacking this fatty acid (e.g. Soudant et al 1997; Aranda-Burgos et al 2014). These animals are able to synthesise DHA by elongation from EPA, this elongation process could help correct DHA deficiency in the diet (Soudant et al 1997). Aranda-Burgos et al (2014) showed that *R. Decussatus* larvae fed a diet based on the EPA, can allocate a part of this polyunsaturated fatty acid for conversion into DHA.

In this study we have seen that the effects of microalgal diets on spat performance appear to be species specific and may also be sensitive to algal culture conditions (Thompson et al 1993; da Costa et al 2017; Svenning et al 2019) which may alter biochemical properties (Aranda-Burgos et al 2014). The culture conditions of all microalgae strains here were rigorously controlled and were the same for all of them, the harvesting phase can also affects the composition of the microalgae (Brown et al 1993; Liang & Mai 2005; da Costa et al 2017), that's why the microalgae were distributed to the spat more or less at the end of the exponential phase and sometimes at the beginning of the stationary phase.

Mixed strains algal diets. In general, the number of strains in the diet influenced growth of spats, and there was a significant difference ($p<0.05$) in growth between the average spat size fed by each type of diet. Results showed that diets with four or more strains supported the most growth in size, followed by diets with three then two strains. Several studies have reported such superiority of mixed diets over single diets (Romberger & Epifanio 1981; Enright et al 1986; Laing & Millican 1986; O'Connor et al 1992; Brown et al 1998; Rivero-Rodríguez et al 2007), this can be explained by the complementarity of growth factors or essential nutrients present in the diets, (Brown et al 1998). This can also be seen in the gap between the average relative instantaneous growth rates within each diet type which becomes increasingly large as we move from complex diets to two-strain diets, for the two-strain diets, only three of ten showed visibly higher relative instantaneous growth rates. Some authors have explained the superiority of mixed diets by the combination of easily digestible and relatively indigestible algae, which increases the residence time of less digestible microalgae in the animal's gut, leading to an increase in the assimilation efficiency of microalgae (Romberger & Epifanio 1981; Brown et al 1998).

Our results showed that the mixed diets with the Tet2 showed the highest values ($p<0.05$) of shell size growth of *C. gigas* spat, indeed out of the 26 mixed diets tested Tet2 was present in the best 13 diets, often associated to Tet1, Cha or both.

In the mixed diets, many performed better than diets composed of the same strains in monostrain mode as reported by Laing and Millican (1986) and explained by

several authors by the synergistic or additive effect of these mixed diets which is due to the balance of nutritional components (Díaz & Martínez 1992 and Velasco-Blanco 1997 in Marquez et al 2019) and other factors such as size and digestibility related to the cell wall structure and morphology of the alga (Le Pennec & Rangel-Davalos 1985; Martínez-Fernández et al 2004). In this study, these were the case for D8 (composed of the two strains Cha and Tet2), D13 (composed of Tet1 with Tet2) and the D9 (composed of the strains Cha and Nan). For the other mixed diets, the effect of adding a second strain was only to reduce spat growth in these regimes: Tet1, Tet2 and Tha. The Cha strain acts negatively on Tha and Tet1 and the Tha strain acts negatively on Tet1 and Tet2, if it is not the toxic effect of these strains which is unlikely - these strains are widely used for aquaculture feed – these effects are due to their low food value or their low digestibility or both, in fact some strains in mixed diets only reduce the nutrient intake of spat (Mohamed Idhalla personal communication). This result can also be seen in the tri-specific diets, out of 10 diets only two have a beneficial additive effect, these are the diet D19 (composed of the three strains Cha, Tet1 and Tet2) and the D21 diet (composed of the three strains Cha, Tet2 and Nan). For the tetra-strains diets only the D29 composed of Cha, Tet1, Tet2 and Nan had a beneficial additive effect, the penta-specific diet did not show an additive effect, the resulting growth of this diet was equal to that of the D4 diet composed only of the Tet2 strain, for this diet adding the other four strains only increased the costs of feeding the spat. In the study by Yang et al (2016), the growth of razor clam *S. constricta* spats fed with two-strain algal diets were lower than those fed single-strain diets.

Regarding survival rates, which is an important factor for commercial oyster spat production (Cochennec-Laureau et al 2011; Mazurié et al 2011), the performance of diets on survival rates differed from those of the instantaneous relative growth rates, the three tanks that produced significant growth performance showed average survival performance. The D8 diet composed of the two strains Cha and Tet2 showed 66±2% of survival rate, the D19 diet composed of the three strains Cha, Tet1 and Tet2 with 60±2% survival rate and the D13 diet composed of Tet1 and Tet2 with 68% survival rate. The result of a study done on mussel larvae *Mytilus galloprovincialis* fed different diets demonstrated no relationship between growth rates and survival rates, the diets that gave the best growth rates were not the ones that gave the highest survival rates (Pettersen et al 2010). However, in this study, the diets that showed the highest survival rates were D7 with 84% survival, D11 and D16 with 82%, in addition, nine other diets showed survival rates higher than 70%. These 12 diets contain in their composition either Tha1 or Cha with Tet1 or Tet2 especially in bispecific diets. Although Tha1 and Cha strains did not support high growth rates, their presence in the diets seems to increase survival rates, this can be explained by the fact that they do not provide a high energy value for growth, but they do present a source of trace elements essential for spat survival. Sakamoto et al (1997) and Pettersen et al (2010) revealed that larval survival was strongly influenced by the proportions of docosahexaenoic acid (DHA).

It was also found that six diets had survival rates of 58% or less, all of which had the Nan strain in their composition. Yang et al (2016) reports a low survival rate (38.1% for small juveniles and 60.1% for large juveniles) was found with one diet having the strain *Nannochloropsis oculata* in its composition. Also, Mohebbi et al (2016) found that *Artemia urmiana* species fed with *N. oculata* showed lower survival rates than those fed with *Tetraselmis sueccia* or *Dunaliella tertiolecta*, which reinforces our results.

Conclusions. For single-strain diets, both Tet1 and Tet2 strains showed the highest spat growth performance, while the Cha strain resulted in the highest survival rates. For the mixed diets, the diets that included the Tet2 strain showed the highest values in terms of growth, as far as survival rates were concerned, there was no relationship between growth rates and survival rates, the diets that showed the highest survival rates contained in their composition either Tha1 or Cha with Tet1 or Tet2 especially in the bispecies diet.

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