Effects of newly isolated microalgae on growth and survival of the Pacific oyster *Crassostrea gigas* spats

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1. Introduction

Hatchery cultures can support marine bivalve farming, supplying spats to extensively farmed populations and improving conditions for delicate life stages throughout production. However, up to half of the production costs of inland bivalve hatcheries and nursery facilities, are used for microalgal production. Five local strains of microalgae were isolated from the Moroccan coast, for their high potential-value to replace the exogenous commercial strains commonly used in bivalve hatcheries. Thus, the aim of this study was to assess the effect of different diets, on the survival and growth of *Crassostrea gigas* spat

2. Materials and methods

2.1. Biological material

- **Microalgal strains**

Five microalgal strains were isolated from several sites along the Moroccan Coastline (Table1), they were cultivated under controlled conditions and used to feed oyster spats (Figure 1).
The biochemical characterization of these strains was also established (Table 1).

Table 1: Characteristics of the microalgae strains used for spats feeding (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)
Size (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)						
ΣMUFA		11.5	-	9.61	34.35	-
18:2 (n-6)	Linoleic acid (LA)	-	19.19	-	-	-
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

- **Oyster spats**

The diploid *Crassostrea gigas* oyster spats (length mean size was 5.91±0.29 mm), Were provided from a commercial hatchery. They were acclimatized to laboratory conditions for one week before beginning the experiment.

Figure 1 : Microalgal cultures.

Figure 2 : Oyster spats.

2.2. Laboratory experiment

- **Experimental design**

Thirty-one experimental feeding diets (Table 2) were tested in tanks of 12 L with 35 oyster spats (feed ration of 4%). Survival (daily) and growth (weekly) were assessed during eight weeks of Feeding.

Figure 3 : Laboratory set up showing the experimental feeding treatments.

Table 2: Compositions and species proportions in ranges 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)

- **Statistics**

One-way ANOVA test was applied to determine differences in growth among different diets (alpha = 0,05).
When ANOVA assumptions were not met, the Kruskal-Wallis test (P < 0.05) was used.

3. Results and discussion

Growth rates demonstrated significant differences among the diets tested (P<0.05) (Figure 1). Significantly higher shell growth rates were recorded in oysters fed multispecific diets compared to the monospecific diets (P<0.05). The maximum rates of shell growth over eight weeks-time under monoalgal diets, was reached by *Tetraselmis sp2* (0,36 mm.wee-1) (Figure 1), however, the plurispecifics diets D8 *(Chae+Tet2)* , and D19 (*Chae+Tet1+Tet2*), gave more increases in size, indeed, the mean size shifted between 5.9 ± 0.04 mm to 11.83 ± 0.14 mm (0,74 mm.week-1) and 11.37 ± 0.1 mm (0,68 mm.week-1) respectively. This is due to the combination of the microalgal species to ensure the full nutritionnal requirements of spats, especially the fatty acid and protein requirements (Elyakoubi et al 2020). The lowests growths of spats were obtained with diets containing *Thalassiosira sp* or *Nannochloropsis sp*, alone, or mixed with other strains.
Regarding survival rate, diets containing either *Thalassiosira sp* or *Chaetoceros sp* with *Tetraselmis sp1* or *Tetraselmis sp2*, especially in bispecific diets showed the highest survival rates (75,2 ± 2 %) (Figure 2). Although, the diets that showed the lowest survival rates (<60 ± 2 %) had the *Nannochloropsis* strain in their composition (Figure 2).

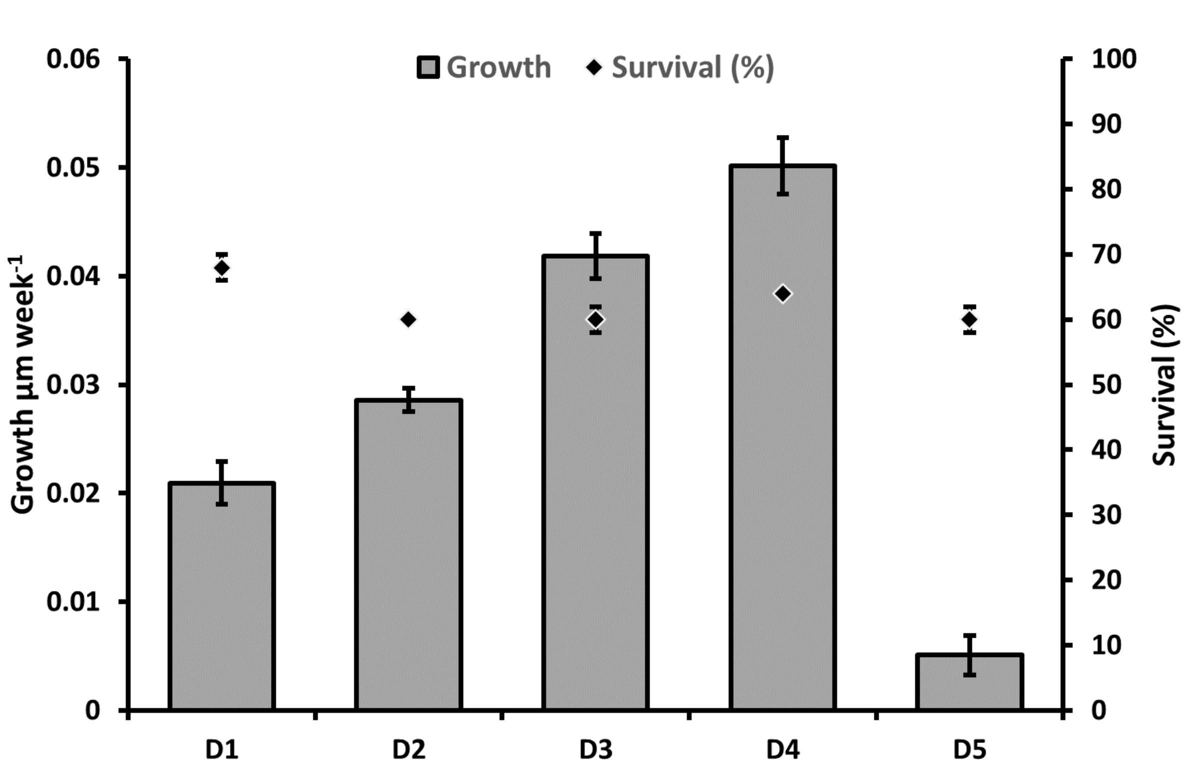

Figure 1: Instantaneous relative growth rate and survival during the feeding experiment.

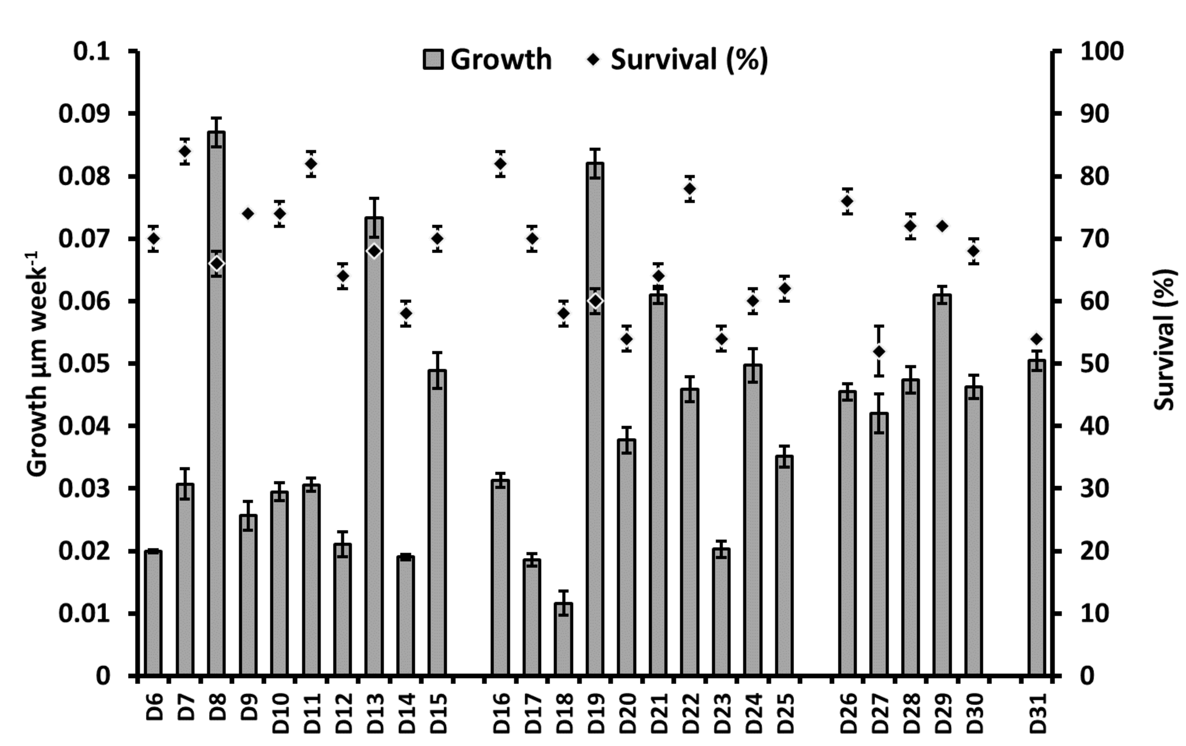

Figure 2: Instantaneous relative growth rate and survival during the feeding experiment

4. Conclusion

The optimal growth and survival of oyster spats were acheived with an equal mixture of *Chaetoceros sp* and *Tetraselmis sp2.* Further studies on physiological pattern (absorption rate and efficiency) could explain better the feeding behavior of oyster spats in captivity.

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