

# Comparative growth and survival of spat of the Caribbean pearl oyster, *Pinctada imbricata* cultivated indoor with microalgae diets and outdoor with natural diet

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## Abstract

We report the results of survival and growth in size and dry mass of spat of the Caribbean pearl oyster *Pinctada imbricata* cultivated under outdoor (field culture) and indoor (Laboratory) conditions. Field group fed on environmental seston. Laboratory groups were fed with mono, binary and ternary mixtures of three cultivated algae: *Isochrysis galbana* (Ig), *Tetraselmis chuii* (Ig) and the *Chaetoceros* sp. (Ch-A, isolated from north-eastern Venezuela). After 30 days of trial, fatty acid profiles of spat were determined along with growth in length and height shell, adductor muscle and soft tissue dry mass. During the field grow-out phase (field culture), samplings were performed at days 1, 15 and 30 to measure environmental variables of phytoplankton biomass (chlorophyll *a*), dissolved oxygen, seston, temperature and salinity. A significant increase in size and soft tissue mass occurred in spat fed the diets including the tropical diatom (*Chaetoceros* sp.). In contrast, monoalgal diets of Tc and Ig yielded no significant differences in size and mass of spat, compared with the field culture. These results suggest that nutritional requirements of cultivated spat for specific fatty acids of physiological importance for marine bivalves, such as: 16:0, 16:1n-7, 18:2n-6, 20:4n-6, 18:3n-3 and 20:5n-3, were satisfied from microalgal diets with Ch-A, alone or in combination, compared with spat fed from the field culture.

**KEY WORDS:** bivalve, growth, highly unsaturated fatty acids, nutritional requirement, spat, survival, tropical area

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## Introduction

In marine bivalves, particularly during the early developing stages (spat), optimal production measured as the growth/survival/cost ratio depends primarily on availability of live algae; nevertheless, the mass culture of microalgae remains as the main constraint (Coutteau & Sorgeloos 1992).

The requirement for live algae for intensive cultivation of bivalves is strongly reduced by rapidly transferring small spat (1–2 mm shell length) from the indoor nursery conditions to the field (Helm 1990; Coutteau & Sorgeloos 1992). Transplantation of small spat to outdoor conditions has been used as a recurrent technique for the grow-out phase, yet it usually yields variable success (mortality and/or poor growth) due to the complex interactions of environmental variables affecting response and condition of the spat (Persoone & Claus 1980), even after transferring all the spat into an optimal combination of temperature and food availability (Claus *et al.* 1983). Pit & Southgate (2000) investigated whether retaining *Pinctada margaritifera* spat in the hatchery for longer periods, prior to transfer to the ocean, would improve growth and survival than spat reared under natural conditions. The hypothesis was rejected because the spat placed in the ocean showed higher growth as a result of superior nutrition available in the natural environment, which led to the conclusion, that the spat should be placed in the ocean as soon as possible.

In general, prior to transferring small spat with a minimum size (1–2 mm), they should be reared in indoor facili-

ties and fed partially, or in some cases exclusively, on phytoplankton. Phytoplankton is the main food source of essential nutritional components, such as sterols and highly unsaturated fatty acids (HUFA), including 20:4n-6 (arachidonic acid, ARA), 20:5n-3 (eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA) (Chu & Greaves 1991; Fernández-Reiriz *et al.* 1999, 2006; Soudant *et al.* 2000). In general, these studies conclude that the family of n-3 fatty acids is essential for the development of bivalve spat. These components vary between species, populations of the same species and culturing conditions. Additionally, criteria such as the acceptability and digestibility of microalgae can be used to explain the nutritional value of a diet (Albentosa *et al.* 1997). Therefore, a precise knowledge of the composition of microalgae species used in indoor or outdoor facilities is essential to provide juvenile bivalves with the correct diet and enhance growth in hard and soft biomass.

The Caribbean pearl oyster *Pinctada imbricata* (Röding 1798) is a promising species for the development of aquaculture in the Caribbean, and on this basis, hatchery production of spat can be necessary (Lovatelli & Sarkis 2011). The spat collection in the field can support a small-scale production (Jimenez *et al.* 2000, Lodeiros & Freitas 2008; Velasco *et al.* 2011), although hatchery production of spat can be necessary to a large-scale production.

In many regions of the Caribbean, including Venezuela, the species is locally consumed and has great potential for the provision of food and pearls (Lodeiros & Freitas 2008; Lodeiros *et al.* 2011). However, very few studies have addressed the importance of spat produced under laboratory conditions, especially to analyse nutritional requirements of larvae and spat for variations on lipid and fatty acid profiles.

This study examines overall increase in total mass and shell length, as well as lipid and fatty acid composition of *P. imbricata* spat fed on different algal species. We compared two species of algae commonly used as diets in bivalve hatcheries (*Tetraselmis chuii* and *Isochrysis galbana*), together with a diatom isolated from the Araya Peninsula, north-eastern Venezuela (*Chaetoceros* sp. strain BGAUDO-35), which has shown rapid growth and easiness of maintenance when cultivated in outdoor facilities (Núñez *et al.* 2002; Lemus *et al.* 2006). The diets were provided in controlled laboratory conditions as mono, binary or ternary mixtures and were compared with the food available from the natural environment (seston).

## Materials and methods

### Experimental design

Wild *P. imbricata* spat were harvested by hand from a natural cohort settled on floating cages used for fish culture in the Charagato Bay, Cubagua Island, Nueva Esparta State, Venezuela (10° 49' 49.17" N; 64° 09' 40.42" O). The young oysters were transferred to a research station in Mochima Bay, Sucre State, Venezuela (10° 20' 47.30" N; 64° 20' 42.10" O) (Fig. 1), in insulated containers packed with moistened foam layers to maintain a cool environment and prevent stress. Thereafter, they were placed during 4 days in polyethylene mesh bags and suspended from a long line at 4 m depth, for acclimatization prior to the trials.

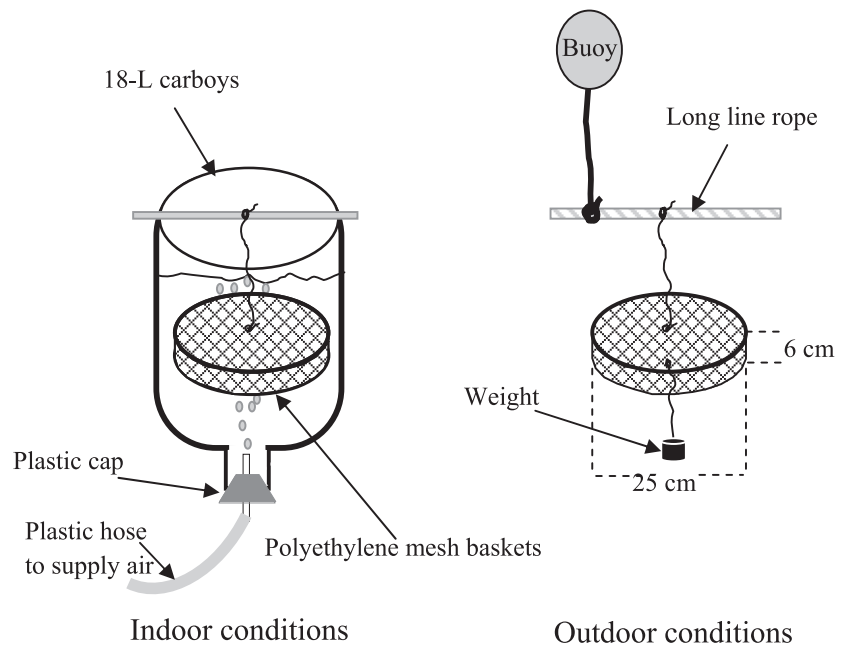
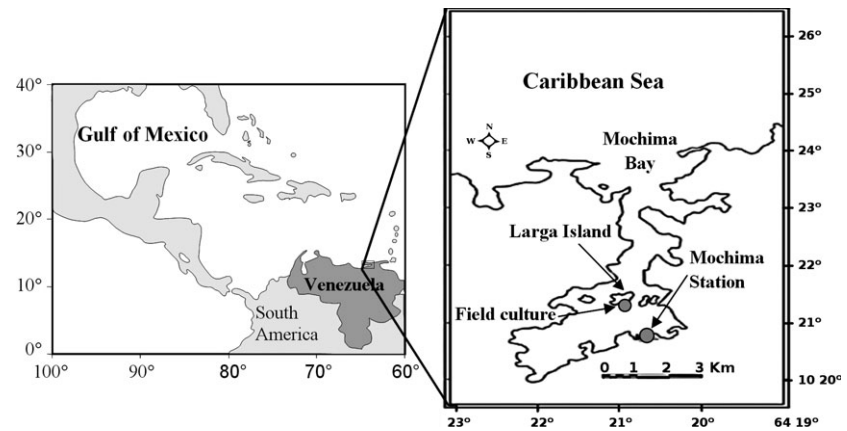
A total of 360 spat with a length and height size of  $10.3 \pm 1.09$  mm and  $8.7 \pm 0.64$  mm, respectively, and tissue dry biomass of  $9.0 \pm 2.5$  mg, were randomly selected and evenly distributed into 24 replicates of 15 oysters. Twenty-one replicates were placed in equal number of 18 L carboys, while the remaining three replicates were fed from the natural ambient at Mochima bay (hereafter referred to as field culture). Triplicate sets of microalgae diets were given to 21 of the replicates (i.e. seven different diets). Selected culture site have a limited regime of currents and waves, because the natural barrier are some small islands (Islas Larga y Redonda) located within the bay (Fig. 1).

All carboys were cut from the bottom and placed in an inverted position, and the mouth was covered with a plastic cap. Each carboy was filled with 7 L filtered (0.5 µm) and UV-sterilized sea water, which was continuously aerated and maintained at a temperature and salinity of  $24 \pm 1$  °C and  $36 \pm 1$ , respectively. The entire seawater volume was fully exchanged daily. For the field culture, the spat were placed in polyethylene mesh baskets (Fig. 2) and suspended from a long line at 4 m depth, near to a mangrove site in the inner area of the Mochima Bay. In the laboratory, the spat were placed in the same type of polyethylene mesh baskets and then introduced into the carboys to try to resemble the conditions of both experimental groups.

### Spat growth and survival

In both laboratory and field culture, the spat were grown for 30 days, and at the end, increases in dry weight of adductor muscle (g) and entire soft tissues (g) were determined for each experimental treatment and replicate. This was done by drying tissue components at 70 °C for 72 h. Increase in shell dimensions (length and height) was deter-

**Figure 1** Study area in north-eastern Venezuela.



**Figure 2** Diagram showing how the spat were cultivated in indoor and field culture.

mined with a digital calliper (0.1 mm precision). Also at the end of the trials, the number of surviving oysters from each triplicate sample belonging to different dietary treatments was used to determine survival with regard to the initial number of spat seeded.

### Dietary treatments

The oyster cultured at field conditions received natural seston as food. Laboratory oysters were fed with mono, binary and ternary mixtures of three cultivated algae. The dietary treatments in laboratory conditions were constituted by the microalgae *I. galbana* (Ig), *Chaetoceros* sp. strain Araya (Ch-A) and *T. chuii* (Tc), belonging to Hapto-

phyceae, Bacillariophyceae and Prasinophyceae class, respectively. *Chaetoceros* sp. (strain BGAUDO-35) is catalogued in the germoplasm collection of Instituto Oceanográfico de Venezuela of Universidad de Oriente as a tropical clone isolated from the Araya Peninsula (Sucre State, Venezuela), whereas *T. chuii* and *I. galbana* are temperate microalgae species that are well adapted to the conditions of our study site (Marín *et al.* 1994). All algal species were supplied as monoalgal diets (Ig, Ch-A and Tc), three binary combinations (Ig + Ch-A, Ig + Tc and Ch-A + Tc) and one ternary mixture (Tc + Ch-A + Ig).

Microalgal strains were cultivated with f/2 medium (Guillard & Ryther 1962), with silica supplement for diatom (*Chaetoceros* sp.), and then grown at the outdoor, in

400-L plastic tanks at a temperature between 24 and 28 °C. Lighting was natural (sunlight) with about 12-h light: 12-h dark photoperiod regime. They were harvested during the final exponential growth phase. The organic dry mass of microalgal cells was calculated by the filtration of a volume of the algal cultures through Whatman GF/C glass fibre filters, previously dried at 80 °C and rinsed with a 0.5-M ammonium formate solution to eliminate salts. Filters were dried to constant weight at 80 °C and ashed to 450 °C in a furnace. Cell concentrations of microalgae cultures were daily determined using a Neubauer chamber. The binary and ternary diets were developed with the dry biomass cell equivalents to obtain equal microalgae components. Daily rations of each diet were calculated taken into account the biomass and size of each algae strain used (Table 1). The daily rations of each mono, binary and ternary diets were estimated as 10% of the initial spat mass (9.0 mg × 15 spat = 135 mg) (Table 2).

### Fatty acids analysis (FA profile)

The nutritional value (FA profile) of algal diets and the spat was determined at the end of the trials. Samples of microalgae and natural seston were taken on a fortnightly basis throughout the trial for biochemical analysis. Samples were centrifuged, freeze-dried and stored at −5 °C until further analysis. Additionally, samples of soft tissues from

**Table 1** Dry biomass (pg cell<sup>−1</sup>) and size (μm) of the three algae species for spat of the Caribbean pearl oyster *Pinctada imbricata* in this study

Microalgae	Dry biomass (pg cell <sup>−1</sup> )	Size (μm)
<i>Chaetoceros</i> sp. strain Araya (Ch-A)	58.9 ± 1.6	5.3 × 7.5
<i>Isochrysis galbana</i> (Ig)	70.7 ± 2.8	5.1 × 3.5
<i>Tetraselmis chuii</i> (Tc)	154.7 ± 1.9	16.1 × 8.2

**Table 2** Daily ration (cell mL<sup>−1</sup>) of the different microalgal diets for spat of the Caribbean pearl oyster *Pinctada imbricata* in this study

Diet	cell mL <sup>−1</sup>	cell mL <sup>−1</sup>	cell mL <sup>−1</sup>
Tc	12 442	—	—
Ig	—	27 163	—
Ch-A	—	—	32 688
Tc + Ch-A	6221	—	16 344
Tc + Ig	6221	13 581	—
Ch-A + Ig	16 344	13 581	—
Tc + Ig + Ch-A	4147	9054	10 896

five spat collected from each experimental replicate were lyophilized and stored at −5 °C. These were treated with a 1 : 2 : 0.6 ratio of a chloroform–methanol–water solution to extract lipids (Bligh & Dyer 1959). The lower phase of the chloroform extract was recovered and evaporated with gaseous nitrogen (N<sub>2</sub>). Lipids were derivatized with a mixture of hydrochloric acid and methanol (HCl : CH<sub>3</sub>OH; 5 : 95 v/v) and heated to 85 °C for 2.5 h (Sato & Murata 1988). The fatty acid methyl esters (FAME) obtained were extracted with hexane (C<sub>6</sub>H<sub>14</sub>, 2 mL), evaporated with N<sub>2</sub>, and resuspended with hexane. Afterwards, the FAME were injected into a gas chromatograph (CP 3800; Agilent Technologies, Santa Clara, CA, USA), adapted with a mass detector (1200; Agilent Technologies) using a capillary column from Omegawax<sup>TM</sup> [Sigma-Aldrich (Bellefonte, PA, USA) with 250 fused silica from Supelco, 30 μm × 0.25 μm × 0.25 μm internal diameter]. FAME were classified as follows: saturated fatty acids without double bonds (SAFA), monounsaturated fatty acids with one double bond (MUFA) and polyunsaturated fatty acids with two or more double bonds (PUFA), including highly unsaturated FA with ≥20 carbons and ≥3 double bonds (HUFA).

### Environmental variables

Temperature was recorded continuously with SEALOG electronic thermographs (Vemco, Halifax) placed at 4 m depth in the same study area in Mochima Bay. Salinity was also measured *in situ* using a hand refractometer ATAGO S/Mill (range 0–100‰). Seawater samples were collected in triplicate with a 5-L Niskin bottle from 4 m above the sea surface. The samples were prefiltered (240 μm) to remove large particulate matter and zooplankton and transferred into a dark container to the laboratory. Seawater samples were filtered through precombusted (450 °C for 4 h) and weighed GF/C filters and later rinsed with isotonic ammonium formate (0.5 M). Total particulate matter (TPM) was established as the weight measured after drying the filters to constant weight at 110 °C for 48 h. Particulate organic matter (POM) corresponded to the weight loss after ignition at 450 °C for 4 h in a furnace. The phytoplankton biomass was estimated using the chlorophyll *a* measured by colorimetric procedures (Strickland & Parsons 1972).

### Statistical analysis

One-way ANOVA, followed by Tukey's test for multiple comparison of means when needed, was used to detect signifi-

cant differences in total increase in soft biomass and height and length shell (significance level of tests was set at  $P < 0.05$ ) and levels of selected FA (14:0, 16:0, 16:1n-7, 18:3n-3, 20:5n-3 and 22:6n-3) as a function of dietary treatments (significance level of tests was set at  $P < 0.001$ ). The homogeneity of variance was determined *a priori* with the Bartlett test. Values of tissue biomass and shell size were log-transformed, and relative percentages of FA were arcsine-transformed to reduce the dependence of variances, normalize the distribution of data and obtain the maximum  $r^2$  value (Zar 1984).

## Results

### Environmental variables of field culture site

Table 3 shows values of different environmental variables recorded during 30 days of experimental cultivation (field culture) of *P. imbricata* spat. Three values corresponding to samplings conducted at the start, halfway and end of the culture period reveal that most environmental parameters varied little during the experimental period. For example, temperature and salinity ranged between 27.01 and 28.45 °C and 37.0 and 38.0, respectively; organic seston, between 0.65 and 1.20 mg L<sup>-1</sup>; inorganic seston, between 3.13 and 5.50 mg L<sup>-1</sup>; and chlorophyll *a*, between 0.95 and 1.51 µg L<sup>-1</sup>. Nevertheless, some values contrast with those obtained in indoor conditions, where temperature was 3–4 °C lower, while chlorophyll *a* of the different diets varied between 18.82 µg L<sup>-1</sup> (Ig diet) and 28.87 µg L<sup>-1</sup> (Tc diet), with a mean between all diets of  $22.57 \pm 3.54$  µg L<sup>-1</sup> being higher than those observed in sea water. Total, inorganic and organic seston of the different diets were not assessed in indoor conditions.

### Survival

At the end of the trials, only one dead spat was registered in the Ig + Tc diet at the end of the trials, but appears to have no relation with the dietary treatments.

### Growth of spat

Increase in length and height size was significantly greater ( $P < 0.05$ ) in spat fed Ch-A, the two binary mixes containing Ch-A (Ch-A + Ig; Ch-A + Tc) and the ternary diet, compared with the spat grown in the field culture, while Tc, Ig and Tc + Ig diet did not show significant differences (Fig. 3a,b). Increase in muscle mass was significantly greater ( $P < 0.05$ ) in spat fed with the unialgal Ch-A diet, the three binary mixtures and the ternary diet (Fig. 4a), compared with the field culture and two of the monoalgal diets (Tc and Ig). Also, increase in soft biomass of remaining tissues of spat receiving microalgae alone or combined (binary and ternary mixes) was significantly higher ( $P < 0.05$ ) than that of spat grown in sea water (Fig. 4b).

### Fatty acids profiles

**Culture microalgae and environmental seston** The FA profiles of the three microalgal diets and seston are shown in Table 4. Higher relative percentages of SAFA occurred for 14:0, 16:0 and 18:0. Seston showed higher percentages of SAFA (53.4%), compared with the Ig diet (34.9%). With regard to  $\Sigma$  MUFA, the major contribution was associated with the Ch-A diet (25.2%), particularly 16:1n-7 (23.7%). The highest contribution of PUFA occurred with the Tc (56.9%) and Ig (42.4%) diets, and the lowest contribution occurred with the seston (29.1%). Highest levels of 20:5n-3 FA occurred in the Ch-A (12.9%) and Tc diets (6.6%), while highest levels of 22:6n-3 occurred in the Ig (6.2%), seawater (4.8%) and Ch-A treatments (4.7%). The  $\Sigma$  of selected FA peaked in the Ch-A (94.28%), Tc (90.24%), Ig (87.73%) and seston (74.85%) (Table 4).

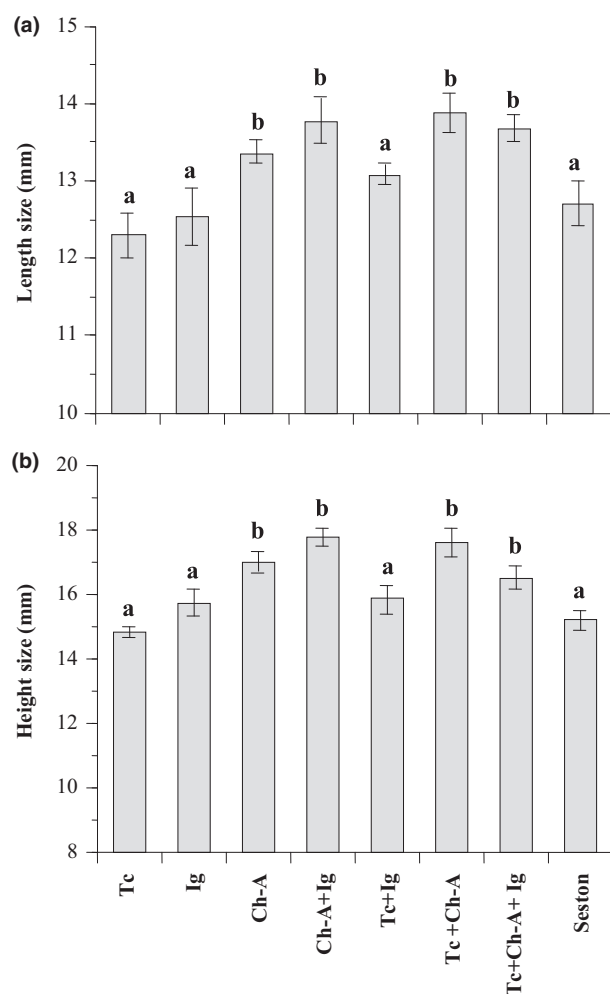
**Spat** The FA profiles of spat fed on different microalgae diets and seston are shown in Table 5. The  $\Sigma$  SAFA in soft tissues showed the highest levels, with values ranging from a minimum of 34.2% in the Tc + Ig diet to a maximum of 44.8% in the field culture. The greatest contribution of FA

**Table 3** Data of different environmental variables recorded during 30 days cultivation of spat of the Caribbean pearl oyster *Pinctada imbricata* (field culture)

Date	Ch-A (µg L <sup>-1</sup> )	Salinity	T. seston (mg L <sup>-1</sup> )	I. seston (mg L <sup>-1</sup> )	O. seston (mg L <sup>-1</sup> )	Temperature (°C)
06/02/08	1.51 ± 0.53	37.00 ± 1.00	6.15 ± 0.25	5.50 ± 0.18	0.65 ± 0.08	28.45 ± 0.22
06/15/08	1.46 ± 0.56	38.00 ± 1.00	4.33 ± 0.15	3.13 ± 0.10	1.20 ± 0.20	27.39 ± 0.40
07/03/08	0.95 ± 0.41	37.00 ± 1.00	6.43 ± 0.23	5.30 ± 0.14	1.13 ± 0.18	27.01 ± 0.32

Ch-A, Chlorophyll *a*; T. seston, total seston; I. seston, inorganic seston; O. seston, organic seston.

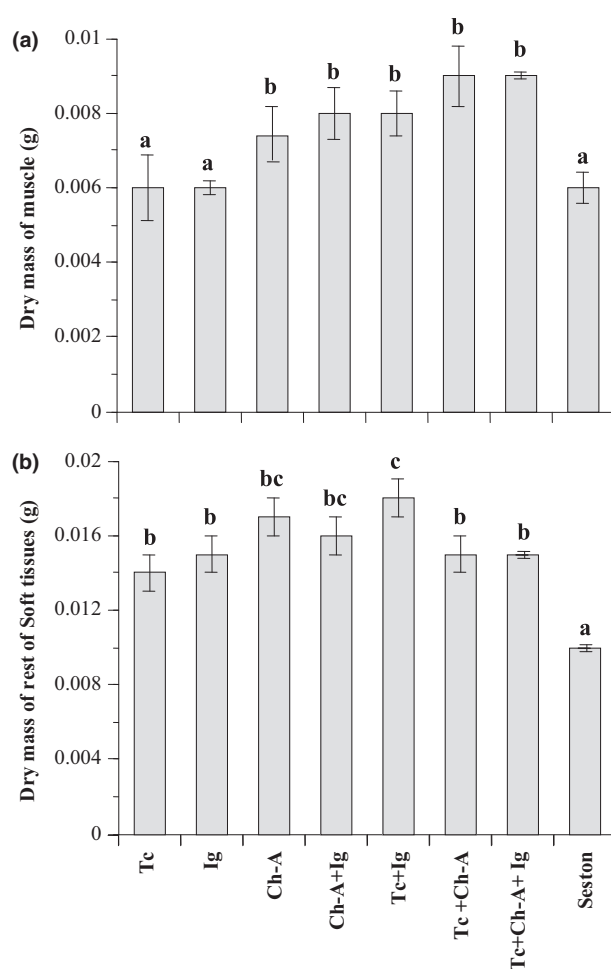




**Figure 3** Mean length and height axis (mm) of spat of the Caribbean pearl oyster *Pinctada imbricata* fed for 30 days on different mixtures of cultivated microalgae and the environmental seston. Different letters above the bars indicate significant differences (Tukey's,  $P < 0.05$ ) among diets.

came from the 14:0, 16:0 and 18:0 FA, followed by the  $\Sigma$  PUFA, with levels between 36.3% (Ch-A diet) and 53.2% (field culture), which also showed high levels of 22:6n-3 (38.1%).

Two groups of PUFA occurred in different proportions; firstly, the  $\Sigma$  PUFA was mainly composed of 18:2n-6, 20:4-6, 18:3n-6, 20:5n-3 and 22:6n-3 FA, whereas maximum levels of 20:5n-3 occurred in spat fed on *Chaetoceros* sp., either Ch-A (17.4%), Ch-A + Ig (15.1%) or TC + Ch-A (18.0%) diets. Secondly, the monoenoic FA showed the highest level of 16:1n-7 from all diets and high levels of 20:5n-3 in spat receiving either the Ch-A (17.4%), Ch-A + Ig (15.0%), Tc + Ch-A (18.0%) or Tc + Ch-A + Ig diets (14.4%).



**Figure 4** Mean muscle and soft tissues mass (g) of spat of the Caribbean pearl oyster *Pinctada imbricata* fed for 30 days on different mixtures of cultivated microalgae and the environmental seston. Different letters above the bars indicate significant differences (Tukey's,  $P < 0.05$ ) among diets.

**Comparison of selected FA profiles between treatments** For SAFA, all spat fed on cultivated microalgae showed significantly lower levels ( $P < 0.05$ ) of 14:0 and 18:0 than spat grown in the field culture. In contrast, spat fed on cultivated microalgae had significantly ( $P < 0.05$ ) higher levels of 16:0, compared with the seston (Table 5).

For monoenoic and n-6 PUFA, values for 16:1n-7, 18:2n-6 and 20:4n-6 were significantly higher in spat fed on cultivated microalgae than with the seston (Table 3), except for the Ch-A (18:2n-6) diets, where the levels were significantly lower (Table 5). Similarly, the n-3 PUFA of spat grown in sea water showed significantly lower levels of 18:3n-3 ( $P < 0.05$ ) and 20:5n-3 FA ( $P < 0.05$ ), compared with spat fed on cultivated microalgae; the exception was the Ch-A diet, where the levels of 18:3n-3 were significantly

**Table 4** Fatty acid profile (%) of the three cultivated microalgae and environmental seston used for feeding spat of the Caribbean pearl oyster *Pinctada imbricata*

Fatty acids (FA)	Ch-A	Ig	Tc	Seston
14:0	10.97 ± 1.15	12.03 ± 0.99	4.10 ± 0.58	14.30 ± 0.28
15:0	1.49 ± 0.47	0.46 ± 0.02	0.53 ± 0.01	2.51 ± 0.38
16:0	11.79 ± 0.38	11.75 ± 0.81	22.21 ± 1.08	12.19 ± 0.99
17:0	0.17 ± 0.02	0.14 ± 0.01	–	2.38 ± 0.15
18:0	7.69 ± 0.23	9.82 ± 0.03	5.40 ± 0.73	17.75 ± 1.53
20:0	0.24 ± 0.03	0.32 ± 0.01	0.28 ± 0.02	1.76 ± 0.14
22:0	0.52 ± 0.06	0.33 ± 0.03	0.03 ± 0.06	1.11 ± 0.04
24:0	0.81 ± 0.08	–	–	1.41 ± 0.19
Σ SAFA	33.68	34.85	32.55	53.41
16:1n-7	23.73 ± 2.75	16.74 ± 0.57	2.60 ± 0.11	5.08 ± 0.32
18:1n-9	1.46 ± 0.02	5.96 ± 0.03	5.33 ± 0.29	4.62 ± 0.32
20:1n-9	–	–	2.17 ± 0.18	1.85 ± 0.14
22:1n-9	–	–	0.40 ± 0.05	5.63 ± 1.49
Σ MUFA	25.19	22.70	10.50	17.18
18:2n-6	13.00 ± 0.11	15.27 ± 0.10	15.74 ± 0.95	13.51 ± 0.04
18:3n-6	1.03 ± 0.09	5.05 ± 0.16	1.02 ± 0.07	3.56 ± 0.03
20:4n-6	6.14 ± 0.89	–	2.62 ± 0.15	–
Σ PUFA n-6	20.17	20.32	19.38	17.07
18:3n-3	3.40 ± 0.04	9.82 ± 0.16	31.00 ± 0.81	5.06 ± 0.17
20:5n-3	12.87 ± 2.52	6.16 ± 0.09	6.57 ± 0.42	2.18 ± 0.26
22:6n-3	4.69 ± 0.01	6.14 ± 0.29	–	4.78 ± 0.41
Σ PUFA n-3	20.96	22.12	37.57	12.02
Σ total PUFA	41.13	42.44	56.95	29.09
n3/n6	1.04	1.08	1.94	0.70
Σ Selected FA	94.28	87.73	90.24	74.85

Ch-A, *Chaetoceros* sp (clone Araya); Ig, *Isochrysis galbana*; Tc, *Tetraselmis chuii*; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SAFA, saturated fatty acids.

Selected fatty acids = 14:0, 16:0, 18:0, 16:1n-7, 18:2n-6, 20:4n-6, 18:3n-3, 20:5n-3 and 22:6n-3.

lower (Table 5). In contrast, the 22:6n-3 FA was significantly lower ( $P < 0.001$ ) in the spat given microalgae diets (alone or combined) than for the spat grown in sea water.

## Discussion

At the end of the trials, only one dead spat was registered in all diets, illustrating that the manipulation associated with the treatments did not produce negative effects in *P. imbricata* spat.

In general, shell size growth of spat fed on cultivated microalgae was greater than in spat grown in the field, except those fed with Tc and Ig diets and their combination. These results suggest that, compared with the seston, the diets containing Ch-A, alone or in combination, probably covered most of the nutritional requirements of actively growing spat, at least for some essential components, such as HUFA.

The pattern of slow growth of spat reared at the field is consistent with findings by Márquez *et al.* (2011) with *P. imbricata* and Mengual *et al.* (2011) with *Pteria colymbus*, who reported slow growth in soft tissue biomass of

young pearl oysters cultivated in different sites of the Mochima Bay in Venezuela. In support to our findings, chlorophyll *a* concentration was relatively low throughout the culture period (field culture), with values ranging between 0.95 and 1.51 µg L<sup>-1</sup>. These values reflecting phytoplankton biomass are similar to those reported at 0–5 m depth in the inner area within the Mochima Bay, ranging from 1.19 mg Chl *a* m<sup>-3</sup> (Marcano *et al.* 2010) to 1.04 mg Chl *a* m<sup>-3</sup> (Salazar *et al.* 2011). It has been reported that the mean annual phytoplankton biomass ranging between <2–3 mg Chl *a* m<sup>-3</sup> may become limited to intensive cultivation of bivalves (Saxby 2002). Nevertheless, food availability differences measured as food concentration could not explain why the spat cultivated at the field yielded lower growth than the spat fed on cultured algae at the laboratory. Although the monoalgal diets Ig and Tc contained the same amount of food (10% of initial spat biomass) as the Ch-A, Ch-A + Ig and Tc + Ch-A diets that promoted significantly higher growth of spat, differences between the former (Ig and Tc) and the spat grown in the field culture are not significant. Given these results, the observed differences in growth patterns should be attribu-

**Table 5** Fatty acid profile (%) of spat of the Caribbean pearl oyster *Pinctada imbricata* fed at the end of the trials with different diets of cultivated microalgae and the environmental seston

Fatty acids (FA)	Tc	Ig	Ch-A	Ch-A + Ig	Tc + Ig	Tc + Ch-A	Tc + Ch-A + Ig	Seston
<b>14:0</b>	<b>5.83 ± 0.09***</b>	<b>9.48 ± 0.61*</b>	<b>6.65 ± 0.91***</b>	<b>7.32 ± 1.54***</b>	<b>4.65 ± 0.47***</b>	<b>6.12 ± 0.25***</b>	<b>7.96 ± 0.71***</b>	<b>12.13 ± 0.30</b>
15:0	1.25 ± 0.16	1.52 ± 0.40	1.01 ± 0.14	0.54 ± 0.12	0.30 ± 0.05	0.41 ± 0.05	0.38 ± 0.02	0.61 ± 0.07
<b>16:0</b>	<b>16.99 ± 1.21**</b>	<b>16.67 ± 1.52**</b>	<b>17.89 ± 1.76***</b>	<b>17.00 ± 2.44***</b>	<b>15.01 ± 1.11*</b>	<b>16.69 ± 1.27**</b>	<b>15.76 ± 0.56*</b>	<b>11.00 ± 1.26</b>
17:0	3.16 ± 0.07	1.09 ± 0.23	1.45 ± 0.12	1.90 ± 0.03	2.91 ± 0.18	1.01 ± 0.08	1.06 ± 0.13	1.60 ± 0.22
<b>18:0</b>	<b>13.01 ± 1.30*</b>	<b>12.41 ± 0.77***</b>	<b>10.88 ± 0.74***</b>	<b>10.05 ± 1.96***</b>	<b>11.21 ± 1.27***</b>	<b>10.14 ± 1.37***</b>	<b>9.58 ± 1.0***</b>	<b>18.16 ± 1.27</b>
20:0	0.90 ± 0.16	0.71 ± 0.04	0.32 ± 0.05	0.29 ± 0.04	0.05 ± 0.02	0.15 ± 0.02	0.24 ± 0.02	1.31 ± 0.12
22:0	0.42 ± 0.06	0.37 ± 0.03	0.25 ± 0.03	—	0.21 ± 0.02	0.16 ± 0.03	0.25 ± 0.03	—
Σ Saturated	41.56	42.25	38.45	37.10	34.34	34.68	35.19	44.81
<b>16:1n-7</b>	<b>4.74 ± 0.15***</b>	<b>14.90 ± 0.85***</b>	<b>22.89 ± 0.42***</b>	<b>17.70 ± 1.75***</b>	<b>9.09 ± 0.15***</b>	<b>16.80 ± 1.24***</b>	<b>16.82 ± 2.41***</b>	<b>1.53 ± 0.40</b>
17:1n-7	1.22 ± 0.27	0.14 ± 0.02	—	—	—	—	—	0.37 ± 0.03
18:1n-9	2.17 ± 0.57	1.95 ± 0.18	0.54 ± 0.04	1.26 ± 0.13	2.21 ± 0.24	1.60 ± 0.17	1.26 ± 0.11	0.99 ± 0.13
20:1n-9	4.77 ± 1.07	2.27 ± 0.25	1.86 ± 0.20	1.70 ± 5.78	1.94 ± 0.18	0.19 ± 0.03	1.51 ± 0.03	—
22:1n-9	1.61 ± 0.05	—	—	—	0.70 ± 0.01	0.36 ± 0.03	0.55 ± 0.06	—
Σ Monounsatur.	14.51	19.23	25.30	20.66	13.94	19.07	19.62	2.89
<b>18:2n-6</b>	<b>7.16 ± 0.06***</b>	<b>8.84 ± 1.46***</b>	<b>2.16 ± 0.03</b>	<b>4.95 ± 0.58***</b>	<b>8.73 ± 0.91***</b>	<b>4.48 ± 0.22**</b>	<b>6.16 ± 0.13***</b>	<b>2.14 ± 0.39</b>
18:3n-6	1.19 ± 0.20	1.16 ± 0.13	1.13 ± 0.10	1.57 ± 0.13	1.51 ± 0.09	1.16 ± 0.09	1.04 ± 0.12	0.37 ± 0.03
20:2n-6	1.31 ± 0.16	1.95 ± 0.14	0.74 ± 0.04	1.24 ± 0.04	1.73 ± 0.30	1.12 ± 0.10	1.54 ± 0.09	2.42 ± 0.04
20:3n-6	1.08 ± 0.27	1.30 ± 0.25	0.91 ± 0.02	1.20 ± 0.19	1.06 ± 0.12	0.86 ± 0.03	1.25 ± 0.04	—
<b>20:4n-6</b>	<b>8.14 ± 2.34***</b>	<b>4.91 ± 0.87</b>	<b>10.61 ± 1.51***</b>	<b>10.57 ± 0.81***</b>	<b>9.16 ± 0.85***</b>	<b>10.82 ± 0.78***</b>	<b>9.81 ± 1.62***</b>	<b>3.27 ± 0.14</b>
Σ PUFA n-6	18.89	18.16	15.55	19.53	22.19	18.44	19.80	8.20
<b>18:3n-3</b>	<b>8.96 ± 0.60***</b>	<b>5.51 ± 0.11***</b>	<b>0.50 ± 0.06*</b>	<b>1.45 ± 0.15*</b>	<b>6.00 ± 0.12***</b>	<b>5.30 ± 0.61***</b>	<b>3.50 ± 0.12***</b>	<b>1.00 ± 0.10</b>
20:3n-3	0.87 ± 0.56	—	—	—	0.11 ± 0.02	0.24 ± 0.02	0.08 ± 0.02	—
<b>20:5n-3</b>	<b>7.24 ± 0.53</b>	<b>6.37 ± 0.44</b>	<b>17.43 ± 1.42***</b>	<b>15.05 ± 0.31***</b>	<b>8.87 ± 1.30**</b>	<b>18.03 ± 1.80***</b>	<b>14.40 ± 0.48***</b>	<b>5.83 ± 0.21</b>
<b>22:6n-3</b>	<b>7.77 ± 1.11***</b>	<b>8.32 ± 1.09***</b>	<b>2.77 ± 0.20***</b>	<b>6.21 ± 0.60***</b>	<b>14.55 ± 0.23***</b>	<b>4.31 ± 0.38***</b>	<b>7.35 ± 0.60***</b>	<b>38.14 ± 4.74</b>
Σ PUFA n-3	24.85	20.20	20.70	22.71	29.53	27.87	25.33	44.97
Σ Total PUFA	43.74	38.36	36.26	42.24	51.72	46.31	45.13	53.17
n-3/n-6	1.32	1.11	1.33	1.16	1.33	1.51	1.27	5.48
Σ Selected FA	79.84	87.41	91.78	90.30	87.27	92.69	91.07	93.20

Tc, *Tetraselmis chuii*; Ig, *Isochrysis galbana*; Ch-A, *Chaetoceros* sp. (clone Araya); PUFA, polyunsaturated fatty acids.The asterisk indicates the significance level (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ) with respect to the environmental seston.

Bold values indicate selected fatty acids.



ted to the quality rather than to the quantity of food (e.g. nutritional essential components, such as HUFA).

This aspect could also be extrapolated to differences in temperature between indoor and field culture, because although temperature was 3–4 °C lower in the laboratory, no significant differences were noted between the observed growth among spat fed with Ig and Tc and the seawater diets. Therefore, it supports the idea that the observed differences in the growth in size and biomass of spat could be attributed to the food nutritional quality of both environments (indoor and sea water).

Fatty acids and FA ratios can be used as biomarkers to characterize seasonal contribution of phytoplankton classes, organic detritus and bacteria in the diet of some bivalve species, such as *Mytilus edulis* (Budge *et al.* 2001; Handa *et al.* 2012). Diatoms are rich sources of 16:1n-7 and 20:5n-3 and yield ratios of  $16:1n-7/16:0 > 1$  and  $20:5n-3/22:6n-3 > 1$  (Budge *et al.* 2001; Dalsgaard *et al.* 2003). In our study, similar ratios occurred in spat fed with the specific Ch-A diet ( $16:1n-7/16:0 = 1.3$ ;  $20:5n-3/22:6n-3 = 6.3$ , respectively), which contrasts with ratios determined for spat grown in the field culture ( $16:1n-7/16:0 = 0.10$ ;  $20:5n-3/22:6n-3 = 0.13$ , respectively). Marine phytoplankton is dominated by the same saturated (16:0), monounsaturated (16:1n-7; 18:1n-7 or 18:1n-9) and polyunsaturated (n-3) FA of 20 and 22 carbons, which is also reflected in bivalves mainly nourished by this food source (De Moreno *et al.* 1976a,b, 1980; Langdon & Waldock 1981; Webb & Chu 1983; Bell & Sargent 1985; Besnard *et al.* 1989; Chu *et al.* 1990). This pattern could explain why the spat fed on cultivated microalgae showed significantly higher levels of 16:0, 16:1n-7, 18:1n-9, 18:3n-3 and 20:5n-3 FA. In particular, the saturated FA 16:0 (Tc, Ig and Ch-A diets) and monounsaturated FA 16:1n-7 (Ig and Ch-A diets) occurred in very high concentrations (see Table 4), whereas highest abundances of polyenoic FA of 20 and 22 carbons were attributed to 20:4n-6, 20:5n-3 and 22:6n-3 FA, respectively. These results are in agreement with findings by De Moreno *et al.* (1980) for the mussel *Mytilus platensis* and by Falk-Petersen *et al.* (2001) for the gastropod and pteropod *Limacina helicina* and *Clione limacina*, respectively. These authors reported that the 16:0 FA was present in greatest abundance in addition to the polyunsaturated 20:5n-3 and 22:6n-3 FA.

Growth in shell height and length of spat fed on diets containing the diatom *Chaetoceros* sp. (strain BGAUDO-35) showed a significant increase in comparison with the spat reared in the field culture. This result, together with the trend of muscle and soft biomass increase with this cultivated alga, illustrates the benefit of the strain over the

environmental seston (field culture). Our findings are supported by previous observations reporting a nutritional advantage of using combined diets compared with unialgal diets, especially if the diet contains the diatom *Chaetoceros* spp. for growing juveniles of the European flat oyster *Ostrea edulis* (Enright *et al.* 1986; Laing & Millican 1986), the pearl oyster *Pinctada mazatlanica* (Saucedo *et al.* 2009) and the lions-paw scallop *Nodipecten subnodosus* (Saucedo *et al.* 2013). In fact, a superior nutritional value of *Chaetoceros calcitrans* and *Chaetoceros muelleri* over other microalgae (e.g. *I. galbana* and *Pavlova salina*) has been reported given the increased growth and performance of juveniles of other bivalve species, such as *O. edulis* (Laing *et al.* 1987), *Saccostrea commercialis* (O'Connor *et al.* 1992), *Crassostrea corteziensis* (Lora-Vilchis *et al.* 2004), *N. subnodosus* (Rivero-Rodríguez *et al.* 2007; Saucedo *et al.* 2013) and *P. mazatlanica* (Saucedo *et al.* 2009). The positive effects of these diatoms may be associated with their high content of EPA, which is ~30% of all FA (Volkman & Brown 2005; Cerón-Ortiz *et al.* 2009). The strain *Chaetoceros* sp. BGAUDO-35 in particular was isolated from our region (Araya Peninsula, north-eastern Venezuela), which could explain better nutritional value and performance when grown in outdoors conditions in the tropics. Additionally, this microalgae clone tends to form short chains or unicellular cells, which represents an important advantage for ingestion and digestion by hatchery-reared early veliger larvae (Lemus *et al.* 2006), as well as for running large-scale culturing activities where production costs may be reduced (Núñez *et al.* 2002). Differences in some physiological indicators of studied bivalves may be related to the thermal tolerance of the microalgae used, particularly if they are of tropical or temperate distribution (Martínez-Fernández *et al.* 2004; Martínez-Fernández & Southgate 2007). Saucedo *et al.* (2013) noted that the tropical *Pavlova salina* shows optimum growth at temperatures near 24–28 °C, but the temperate *Pavlova lutheri* likely experiences thermal stress near to these temperatures.

It could be expected that spat grown in the field culture, where there is a multitude of microalgae species, provided different FA profiles and, ultimately, a greater nutritional balance (synergism). However, we did not observe this condition, and the sum of selected FA reached 93.20%, with an important contribution (44.81%) coming from the sum of the 14:0, 16:0 and 18:0 FA (Table 2). The relative high percentages of these FA observed in spat grown in the field culture suggest that they fed on others food sources, such as organic detritus and/or bacteria (Chuecas & Riley 1969; Perry *et al.* 1979; Budge *et al.* 2001; Dalsgaard *et al.* 2003).

Cranford & Grant (1990) reported that, although phytoplankton is an important food source for bivalves, organic detritus could contribute to the energy demand in periods when phytoplankton concentrations are low. Similar results highlight the importance of POM concentration in comparative trials where differences between culture locations of some bivalve species were tested (MacDonald & Thompson 1985; Wallace & Reinsnes 1985; MacDonald & Bourne 1987; Wilson 1987; Toro *et al.* 1995; Kleinman *et al.* 1996; Freitas *et al.* 2003, 2010).

Overall, the value of the different FA is given as the relative percentage of some selected FA, which significantly decreased in spat grown in the field culture, including those FA of known energetic importance for marine bivalves (16:0, 16:1n-7, 18:2n-6, 18:3n-3 and 20:5n-3; see Table 5). In terms of essential FA, these results could also be attributed to poor quality of food at the site where the oysters were placed, which would also explain the low levels of the FA referred above, the high relative percentages of 18:0 (18.16%) and 22:6n-3 FA (38.14%) due to predominantly structural function (Pazos *et al.* 1997; Labarta *et al.* 1999; Bergé & Barnathan 2005), and their specific retention in soft tissues of spat grown in sea water.

In summary, we observed a significant increase in shell size, muscle mass and soft tissue mass of spat fed on cultivated microalgal diets, especially those including the diatom Ch-A, when compared to those grown at the field or fed monoalgal diets (Ig and Tc). In tropical areas, such as the Caribbean, we recommend using native microalgae as main diet for cultivating bivalves and other invertebrates, due to lower production costs in infrastructure and electric energy (e.g. air conditioning equipment is not necessary). Although *Chaetoceros* sp. BGAUDO-35 may be identified as *C. muelleri*, we recommend conducting taxonomic and biomolecular studies to confirm the exact position, likely as the *Chaetoceros* sp (clone Araya), within the Bacillariophyceae class.

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