Research Article

Isolation, culture and evaluation of *Chaetoceros muelleri* from the Caribbean as food for the native scallops, *Argopecten nucleus* and *Nodipecten nodosus*

Luz Adriana Velasco[1], Silvia Carrera[1] & Judith Barros[1]
Laboratorio de Moluscos y Microalgas, Universidad del Magdalena
Taganga, Santa Marta, Colombia
Corresponding author: Luz A. Velasco (molmarcol@gmail.com)

ABSTRACT. The potential of the Caribbean strain *Chaetoceros muelleri* (CHA-C-04) to be produced and used as diet of two commercially important native scallops (*Argopecten nucleus* and *Nodipecten nodosus)* was assessed, using the non-indigenous *Chaetoceros calcitrans* strain, as a control. Growth and biomass of both diatoms were compared under different culture environments (indoor and outdoor) and culture media (F/2, organic fertilizer triple 15 and humus extract). In addition, their bromatological composition and effect on the physiological condition of the scallops fed with both diatoms were compared. The growth and biomass production of *C. muelleri* and *C. calcitrans* were higher under indoor conditions and using the F/2 media. Although the content of proteins, lipids and energy was higher in *C. muelleri* than in *C. calcitrans*, its size, organic content, growth and biomass values were not different. Scallops fed with both diatoms strains shows similar values for all of the physiological variables measured, including rates of absorption, oxygen consumption, ammonia excretion and scope for growth. Results suggest that the local strain *C. muelleri* can be successfully produced and used in the diet of *A. nucleus* and *N. nodosus*, but do not offer productive advantages.

Keywords: *Chaetoceros*, bivalves, physiology, nutritive value, microalgae culture, aquaculture.

Aislamiento, cultivo y evaluación de *Chaetoceros muelleri* del Caribe como alimento para los pectínidos nativos, *Argopecten nucleus* y *Nodipecten nodosus*

RESUMEN. Se evalúa el potencial de la cepa del Caribe *Chaetoceros muelleri* (CHA-C-04) para ser producida y utilizada como dieta de dos pectínidos nativos de importancia comercial (*Argopecten nucleus* y *Nodipecten nodosus*) usando la cepa foránea *Chaetoceros calcitrans* como control. El crecimiento y biomasa de ambas diatomeas se compararon en diferentes ambientes (interior y exterior) y medios de cultivo (F/2, fertilizante orgánico triple 15 y extracto de humus). Además, se comparó su composición bromatológica y efecto sobre la condición fisiológica de los pectínidos alimentados con ambas diatomeas. El crecimiento y la producción de biomasa de *C. muelleri* y *C. calcitrans* fueron superiores en ambientes cerrados y usando el medio F/2. Aunque el contenido de proteínas, lípidos y energía fue mayor en *C. muelleri* que en *C. calcitrans*, sus valores de tamaño, contenido orgánico, crecimiento y biomasa no fueron diferentes. Los pectínidos alimentados con ambas cepas presentaron valores similares para todas las variables fisiológicas medidas, incluyendo las tasas de absorción, consumo de oxígeno, excreción de amonio y crecimiento potencial. Los resultados sugieren que la cepa local *C. muelleri* puede ser producida con éxito y utilizada en la dieta de *A. nucleus* y *N. nodosus*, pero no ofrece ventajas productivas.

Palabras clave: *Chaetoceros*, bivalvos, fisiología, valor nutritivo, cultivo de microalgas, acuicultura.

INTRODUCTION

Alive planktonic diatoms are essential components in the diets supplied to bivalve mollusks in hatchery conditions (Albentosa *et al*., 1997; Ponis *et al*., 2003). Among the most used and nutritive diatoms are *Chae-* *toceros calcitrans* (Paulsen) Takano and *C. muelleri* Lemmermann 1898 (Brown *et al*., 1997; Martínez-Fernández *et al*., 2004; Cerón-Ortiz *et al*., 2009; Liu *et al*., 2009; Petersen *et al*., 2010; Ragg *et al*., 2010), which have been isolated from the eastern Pacific or the north-eastern Atlantic, respectively. Those strains are

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commonly imported by the hatcheries around the world, which could present some problems such as the risk of species introduction into natural ecosystems (De Pauw *et al.,* 1984; Andersen & Kawachi, 2005), lower production parameters due to lack of adaptation to local environmental conditions (Andersen & Kawachi, 2005) and/or lesser nutritional quality for the local bivalves (Brown *et al.*, 1998; Gouda *et al.*, 2006).

Argopecten nucleus and *Nodipecten nodosus* are two Caribbean commercially important scallop species which have been produced in hatchery using as food the traditional strains *C. calcitrans* and *Isochrysis galbana* (Velasco, 2008). Nevertheless, higher values of growth and reproductive conditioning of *A. nucleus* and *N. nodosus* has been obtained in sea suspended culture instead in hatchery conditions and it has been attributed to the higher nutritional quality of local microalgae and/or a higher diversity of food items (Rupp *et al.*, 2005; Velasco & Barros, 2007, 2009; Velasco, 2008). Then, the use of local strains as food for the Caribbean scallops could be more suited considering environmental, productive and/or nutritional parameters.

Although there are some studies about isolating, culture and nutritional evaluation of local marine microalgae strains as food for bivalves (Ewart & Epifanio, 1981; Brown *et al.*, 1998; Knuckey *et al.*, 2002; Gouda *et al.*, 2006). Local marine microalgae strains are not easily available in the Caribbean zone and there are only a few studies about its isolation and/or culture (Bermúdez *et al.*, 2002; Angarita & Sánchez, 2003; Prieto *et al.*, 2005), but there are no published studies about their nutritional evaluation for bivalves. In order to use local microalgae strains as food in aquaculture it is necessary to isolate them and find ways to produce high biomass using simple and inexpensive techniques.

Culture media and environment of culture are among the principal factors affecting the microalgal growth and the production costs (Coutteau & Sorgeloos, 1992; Borowitzka, 1999; López-Elías *et al.*, 2005; Banerjee *et al.*, 2011; Lananan *et al.*, 2013). There are a variety of suitable culture media, including enriched seawater media (Walne, 1966; Guillard, 1975; Harrison *et al.*, 1980) or synthetic media (*i.e.*, ASW, Algal-1). Nevertheless, their use is highly expensive in massive microalgae cultures still using commercial grade reagents (López-Elías & Voltonina, 1993). Alternative media such as agricultural fertilizers extracts of soils or macrophytes and/or treated waste waters have been used with different results (Fabregas *et al.*, 1987; Sánchez-Saavedra & Voltolina, 1994; Nieves *et al.*, 1996, 2000; Godínez *et al.*, 2000; Valenzuela-Espinoza *et al.*, 2002). On the other hand, in tropical and subtropical countries microalgae may be

grown at indoor conditions, under relatively stable conditions, or at outdoor environments, under variable temperature and irradiance conditions, and at a lower cost (Borowitzka, 1999; López-Elías *et al.*, 2003, 2005).

Direct determination of the microalgae value on the reproductive conditioning and/or growth of bivalves require numerous long-term experiments which place important demands on physical facilities, time, labor, and economic resources. The use of physiological measurements is an alternative method for estimating comparative value among diets, with greater simplicity and in less time, as well as providing more information on the factors responsible of the organism's responses (Widdows, 1985a). The scope for growth is very precise and sensible index of stress conditions when the measurements are made carefully (Widdows, 1985a; Grant & Cranford, 1991) which is positively correlated with the bivalve growth rate (Bayne *et al.*, 1979; Riisgård & Randløv, 1981) and gonadic ripeness (MacDonald & Bourne, 1987; Navarro *et al.*, 2000). This index has been used successfully in *A. nucleus* and *N. nodosus* in order to select the appropriate temperature and diet hatchery conditions (Velasco, 2006, 2007).

This study was performed in order to evaluate the value of a Colombian Caribbean microalgae strain to be used as live food for native commercially important scallops. First, the strain *C. muelleri* Lemmermann, 1898 (CHA-C-04) was isolated in the Colombian Caribbean. Then, its growth and biomass production were assessed under different culture conditions, using the traditional strain, *C. calcitrans,* as a control. Finally, we analyze their bromatological composition and compared the physiological responses of the scallops, *A. nucleus* and *N. nodosus*, fed with both microalgal strains.

MATERIALS AND METHODS

Microalgae isolation

The local microalgae strain were obtained from phytoplankton samples, extracted on March 24 2004 (2-4 pm), by means of 3 sub-superficial haulages with a mesh of 56 µm in the Bay of Taganga, Santa Marta, Colombia (11°16′03″N, 74°11′24″W). In this region water temperatures are between 26 and 31ºC, salinities between 32 and 36 (Velasco & Barros 2007, 2009; Velasco *et al.*, 2009a). Phytoplankton samples were located in 200 mL flasks and translated to the Laboratorio de Moluscos y Microalgas of the Universidad de Magdalena, Taganga (11°16'03"N, 74°11'24"W). They were diluted with microfiltered water (1 µm), sieved using 10 µm mesh sizes, enriched

with the culture media F/2 (Guillard, 1975), maintained indoor in glass flasks (500 mL) with constant fluorescent illumination (45 µmol quanta m^{-2} s^{-1}), at 24ºC and salinity of 35. The diatom, *C. muelleri*, was isolated after 45 days of this mixed cultivation using the techniques of serial dilutions and streaking in plates (Guillard, 1973; Hoshaw & Rosowski, 1973). Scanning electron microscopy (SEM) was used to identify the diatom isolated. Three samples of *C. muelleri* cultures (10 mL) were concentrated and rinsed with a tamponed sodium phosphate solution (pH = 7.3) by centrifugation. Samples were fixed adding aldehyde glutamate (2.5%). Fixed cells were rinsed with distilled water, dehydrated in ethanol (30, 50, 70 and 100%) and mounted on stubs. Mounted samples were dried by critical point drying and sputter coated with gold before viewing using a FEI SEM Quanta 200-r. Diatom species were identified following the keys in Hustedt (1930) and Rines & Hargraves (1988). The *C. calcitrans* (Paulsen) strain was obtained from the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), México.

Microalgae culture experiments

A factorial culture experiment (2x2x3) was done with two microalgal strains: a) Caribbean strain, *C. muelleri*, and b) traditional strain, *C. calcitrans*; testing two culture environments: a) indoor at 24 ± 0.5ºC, and b) outdoor at 28 ± 2ºC; and three culture media: a) Guillard f media (F/2; Guillard 1975), b) triple 15 (T15; Nutrimon®, Monomeros, Colombia), and c) humus extract (H; Nutrimon®, Monomeros, Colombia). All of those 12 treatments were realized by triplicate using 200 mL glasses containers, water at 35 of salinity, compressed air injected and constant fluorescent illumination of 45 µmol quanta m^{-2} s^{-1} and initial density of 0.6x10^3 cell µL^{-1}. The outdoor treatments were exposed to the same conditions, excepting at the daytime, when artificial lighting were replaced by the solar irradiance, with values as high as 160 µmol quanta m^{-2} s^{-1}. Appropriated quantities of T15 and H were diluted in distilled water, sterilized (15 PSI per 60 min) and microfiltered (1 µm), in order to prepare the stock solutions for media culture with the same total nitrogen of the F/2 stock solution (75 mg L^{-1}; Guillard, 1975). The composition of each medium is presented in Table 1.

The same parental exponential phase culture maintained in the experimental conditions for two generations was used as inoculums in each one of the treatments. Cellular density was determined daily from three samples (1 mL) collected at the same hour (4 pm) from each of the replica after homogenization. Optical analysis was performed using a Neubaeur plate (0.1 mm of deep) under a microscope.

Table 1. Composition of media cultures tested. Guillard f media (F/2; Guillard, 1975), triple15 (T15; Nutrimon®, Monomeros) and humus extract (H; Nutrimon®, Monomeros).

Constituents	Formula	F/2 (%)	H (%)	T15 (%)
Sodium nitrate	NaNO$_3$	63.69	40.10	-
Ammonia nitrogen	NH$_4^+$	-	-	22.90
Nitric nitrogen	NO$_3^-$	-	-	10.44
Sodium phosphate	NaH$_2$PO$_4$	4.25	5.15	-
Phosphoric oxide	P$_2$O$_5$	-	-	33.33
Potassium	K	-	33.70	-
Potassium oxide	K$_2$O			33.33
Sodium silicate	Na$_2$SiO$_3$	25.48	-	-
Cupric sulfate	CuSO$_4$.5H$_2$O	0.01	-	-
Zinc sulfate	ZnSO$_4$.7H$_2$O	0.02	-	-
Cobalt chloride	CoCla.6H$_2$O	0.01	-	-
Manganese chloride	MnCl$_2$.4H$_2$O	0.149	-	-
Sodium molybdate	Na$_2$MoO$_4$.2H$_2$O	0.01	-	-
Ferric chloride	FeCl$_3$.6H$_2$O	2.65	-	-
Disodium EDTA	Na$_2$EDTA	3.70	-	-
Biotin	C$_{10}$H$_{16}$N$_2$O$_3$S	0.001	-	-
Cyanocobalamin	C$_{63}$H$_{58}$CoN$_{14}$P	0.01	-	-
Thiamine	C$_{12}$H$_{17}$ClN$_4$OS	0.02	-	-
Magnesium	Mg	-	20.45	-
Boron	B	-	0.6	-
	Relation N:P	15:1	8:1	1:1

$$D\ (cel\ mL^{-1}) = FD \times C \times 10,000\ mL^{-1}$$

where D: cell density, DF: dilution factor and C: media number of cells in each quadrant.

Culture volume lost in each replica by effect of sampling and evaporation was replaced daily with distilled and sterilized water. Specific growth rate (K) was calculated using the equation proposed by Fogg (1965):

$$K = (LnNf - LnNi) / t$$

where N is the initial (i) and final (f) cellular density and t is the duration of the culture until the stationary phase.

Dry biomass of each replica was determined from a daily culture sample (10 mL) filtered on a glass fiber filter (1.5 µm) and dried at 70ºC for 24 h, following the gravimetric method described by Strickland & Parsons (1972).

Microalgae characterization

Bromatological composition of both diatoms were determined from triplicate cultures (3 L), using F/2 medium, under the same indoor conditions described above, and using an initial density of 0.2x10^3 cell µL^{-1}. Three samples of each microalgae culture (500 mL) in exponential phase (4 days) were analyzed in terms of cell diameter by microscopy, total particulate matter (TPM) and organic content (POM) following the gravimetric method described by Strickland & Parsons (1972). Each subsample was filtered on glass-fiber filters (diameter = 0.45 µm; Millipore) which had been previously washed

with distilled water, ashed at 450°C for 4 h, and weighed. The filtrate was washed with 3% ammonium formate, dried at 70°C for 48 h, and weighed; and finally the filters were ashed at 450°C for 4 h and re-weighed. Protein quantification was carried out according to Lowry *et al*. (1951), total lipids using the protocol of Dubinsky (1979) and total carbohydrates was calculated from the difference between organic matter and the sum of protein and lipid content (AOAC, 1995). The energetic content of the diets was estimated from three samples (0.5 g dry weight) of each strain using a micro-calorimeter (IKA® C200, precision 0.0001 J mg^{-1}).

Bivalve physiological measurements

About 30 specimens of *Argopecten nucleus* (length 40 ± 0.1 mm and dry tissue weight 0.93 ± 0.2 g) and 30 specimens of *Nodipecten nodosus* (length 83 + 0.8 mm and dry tissue weight 5.55 + 1.6 g) were obtained at the INVEMAR bivalve culture station at Neguanje Bay (11º20'N, 74.05'W), in the Tayrona National Natural Park (PNNT), Colombia. The scallops were transported in humid condition to the Laboratorio de Moluscos y Microalgas where their shells were cleaned of encrustations and each individual was marked for identification. Acclimation to laboratory conditions was achieved by holding the scallops in an aerated 250 L seawater tank for one week at 25°C and salinity of 36, while feeding them a microalgal suspension of laboratory-cultured *Isochrysis galbana* and *C. calcitrans* (1:1) at a rate of 3% (dry biomass) of their dry body weight daily.

Two dietary treatments with monoalgal cultures of *C. muelleri* and *C. calcitrans* were tested. Each experimental diet was administered under constant conditions of temperature (25°C), salinity (35), and microalgae concentration, a number of cells equivalent to 4.36 ± 0.08 mg L^{-1} of dry weight (17.3 ± 0.4x10^6 cells of *C. calcitrans* per mL and 13.1 ± 0.7x10^6 cells of *C. muelleri* per mL). Seven test scallops were selected haphazardly for each feeding trial, and the trials were run for 15 h, which included 12 h for feeding determinations and 2 h for the oxygen consumption and excretion measuring.

The experimental diets were administered using a flow-through system consisting of 16 chambers (0.8 L for *A. nucleus* and 1.6 L for *N. nodosus*), designed following Riisgård (1977). A constant flow (150 ± 10 mL min^{-1}) of the experimental diet was directed by gravity from a mixing tank into each chamber; 14 chambers were used for individual bivalves (7 individuals of each species) and two chambers contained empty valves which served as controls. Valve opening by test specimens was continually observed and individuals which failed to open normally were eliminated from the experiment.

The experimental diets were prepared by mixing appropriate volumes of 1 μm microfiltered seawater and microalgae which had been cultured in Guillard F/2 medium (Guillard, 1975) at 24ºC and the conditions of light and salinity previously described, and used in the exponential phase.

The absorption rate (AR) was determined by the biodeposition method described by Iglesias *et al*. (1998), validated by Navarro & Velasco (2003), using the 'flow-through chamber method' described by Riisgård (2001). Feces were quantitatively collected every hour using Pasteur pipettes. The mass and organic content of the feces produced by each test bivalve were estimated separately using the gravimetric method described above for the diet samples.

Oxygen consumption (OCR: mL O$_2$ h^{-1}) and ammonium excretion (UR: μg NH$_4$-N h^{-1}) of both scallops were determined for each treatment on just fed animals by placing them in individual chambers (0.8 and 3 L for *A. nucleus* y *N. nodosus*, respectively) after rinsing the chambers with a 25% HCl and filling with <1 μm filtered and aired seawater. Chambers were sealed and incubated for 2 h at the same temperature at which they were fed, alongside a control chamber devoid of specimens. Then two water samples were taken from each experimental chamber to determine oxygen consumption rates and rates of excretion. Oxygen consumption was never measured at ambient oxygen tension lower than 70% saturation. Oxygen concentration was estimated following the Winkler method as modified by Carritt & Carpenter (Strickland & Parsons, 1972) and ammonia excretion was determined by the phenol-hypochlorite method (Widdows, 1985b).

Scope for growth (SFG) was calculated from the equation given by Widdows (1985a) after converting all the physiological rates to energy equivalents (J h^{-1}):

$$SFG\ (J\ h^{-1}) = A - (R + U)$$

where A = energy absorbed (J h^{-1}) = AR mg h^{-1} x energy content of each microalgae J mg^{-1} (Table 2), R = oxygen consumption (J h^{-1}) = OCR mL O$_2$ x 20.08 J (Gnaiger, 1983), U = ammonium excretion (J h^{-1}) = UR mg NH$_4$-N h^{-1} x 24.8 J (Elliot & Davison, 1975).

The physiological rates were converted to a standard individual of 1 g dry tissue weight and with a macroscopic gonadal stage of I (immature animals). For this, the soft tissues were dried at 70°C for 48 h, and then individually weighed. Standardization employed the equation of Bayne *et al*. (1987):

$$Yst = (1\ /\ We)^b\ x\ Ye$$

Table 2. Characterization of *Chaetoceros muelleri* and *C. calcitrans* and one-way ANOVA analyses results. Values are means ± standard error. Different superscripts indicate significant differences between species.

	Chaetoceros calcitrans	*Chaetoceros muelleri*	F	P
Particulate organic matter (%)	81.4 ± 6.0^a	79.7 ± 12.0^a	0.04	0.8427
Size (µm)	5.0 ± 2^a	5.5 ± 1.5^a	0.99	0.3500
Proteins (%)	40.3 ± 0.8^b	59.0 ± 0.9^a	17.31	0.0141
Lipids (%)	23.0 ± 1.4^b	31.0 ± 1.3^a	23.06	0.0086
Carbohydrates (%)	37.0 ± 1.1^a	10.0 ± 0.9^b	32.80	0.0000
Energetic content (J mg^{-1})	22.9 ± 0.4^b	25.1 ± 0.3^a	58.08	0.0016

where Yts = standardized physiological rate, Ye = non standardized physiological rate, We = experimental animal weight (g), b = dependence of the physiological rate to the size of the animals. b values used were those determined previously for the studied scallops (Velasco, 2007).

Statistical analysis

Factorial repeated measures ANOVA was applied to determine the existence of significant differences of growth and biomass production of the microalgae strains among factors (species, media culture and environment culture). One-way ANOVA analyses were carried out to compare the bromatological contents of the microalgae strains as well as the scallop's physiological variables fed with both diatoms. Tests for normality (Kolmogorov-Smirnov) and homoscedasticity (C of Cochran) were carried out on all dependent variables. Excretion rates were ln transformed, and absorption rates, oxygen consumption rate and scope for growth were transformed to square roots. The statistical analyses were carried out following Zar (1999) considerations and using Statgraphics-plus 5.0® and IBM SPSS 20 software, with a 0.05 alpha for the decisions.

RESULTS

Microalgae characterization

Cells of *Chaetoceros muelleri* (CHA-C-04) are similar to *Chaetoceros calcitrans* with rectangular shape in girdle view and elliptical shape in valve view. But local strain is slightly larger (between 4 and 9 µm), setae are longer (two times the pervalvar length) and straighter. *C. muelleri* presented significantly higher values of proteins, lipids and energy in comparison with *C. calcitrans*, but lower content of carbohydrates. The organic content, as well as the size of both microalgae were statistically similar.

Microalgae culture experiment

C. muelleri cultures with initial values of 0.6x10^3 cell µL^{-1} and 0.3 mg mL^{-1} obtained densities as high as 8.7x10^3 cell µL^{-1}, as well as maximum biomasses of 3.8 mg mL^{-1}, in 6 days (Fig. 1). While *C. calcitrans* cultures with initial densities of 0.6x10^3 cell µL^{-1} and biomasses of 0.3 mg mL^{-1}, after 7 days reached values of 8.5x10^3 cell µL^{-1} and 3.7 mg mL^{-1}, respectively (Fig. 2). Specific growth rate (K) was among 0.14 and 0.38 div day^{-1} in *C. muelleri* (Fig. 1), and between 0.07 and 0.32 div day^{-1} in *C. calcitrans* (Fig. 2). Excepting in H treatments, the density and biomass of *C. muelleri* and *C. calcitrans* had an exponential increase along the first six days of the culture (Figs. 1-2); after that, the values were stabilized; and finally, they decreased (between days 7 and 10 of the culture). None of the H treatments presented any significant increment in density nor in biomass ($P = 1.00$).

Repeated measures ANOVA analyses showed that the density and biomass of *C. muelleri* and *C. calcitrans* (df = 1; F = 0.33; $P = 0.5765$) were statistically similar. Additionally, they demonstrated a significant effect of the culture environment (df = 1; F = 13.33; $P = 0.0011$) and media (df = 2; F = 39.36; $P = 0.0000$), and the interaction of both factors on the growth parameters. The density and biomass of *C. muelleri* maintained in F/2 were significantly higher than those of the other media ($P < 0.001$); while the values presented in the H media were significantly lower ($P < 0.001$). Both species growth was significantly higher under indoor conditions in comparison with outdoor treatments ($P < 0.003$), except in the humus as well as in the *C. muelleri* with T15 treatments, where the culture environments did not affect growth parameters ($P > 0.063$).

Bivalve physiological analysis

The AR of *Argopecten nucleus* and *Nodipecten nodosus* were between 6.1 and 20.6 mg h^{-1} g^{-1} (Fig. 3a), OCR among 0.54 and 1.95 mL O_2 h^{-1} g^{-1} (Fig. 3b), and UR varied between 126.3 and 296.7 µg NH_3-H h^{-1} g^{-1} (Fig. 3c). SFG values were between 107.1 and 456.2 J h^{-1} g^{-1} (Fig. 3d). The type of microalgae strain does not have a significant effect over the AR (df = 1; F = 0.02; $P > 0.8962$), OCR (df = 1; F = 3.68; $P = 0.0686$), UR (df = 1; F = 1.37; $P = 0.2543$) and SFG (df = 1; F = 0.01; $P = 0.9437$).
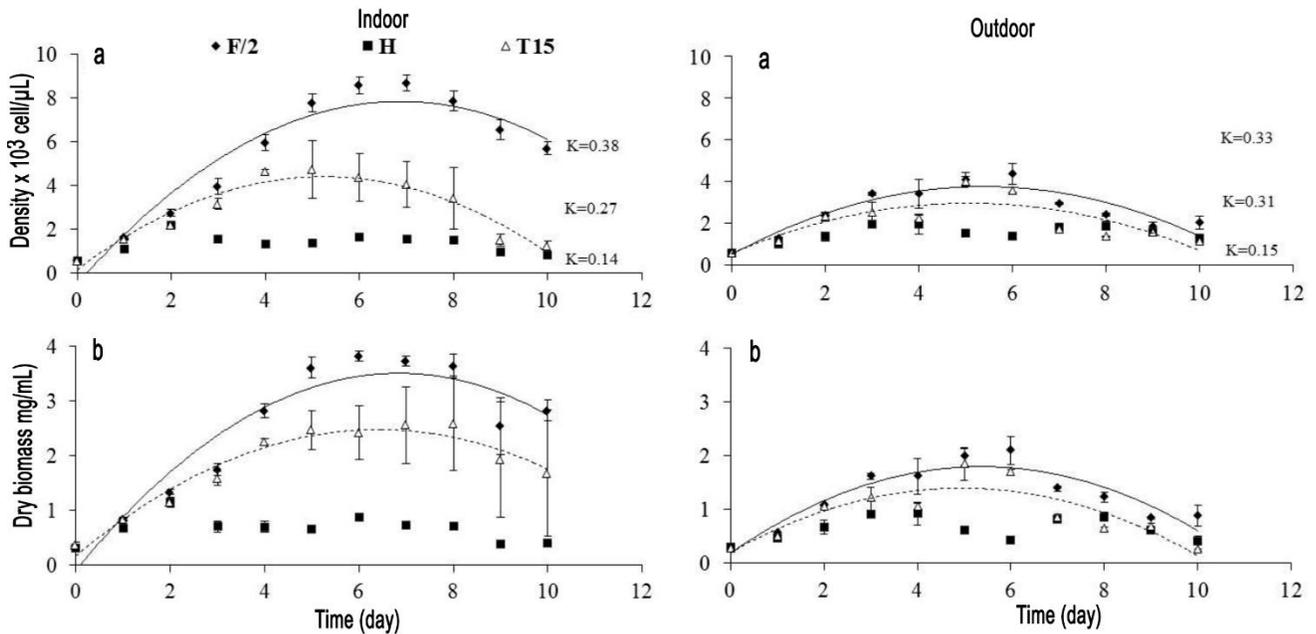
Figure 1. a) Growth in cell density and b) Biomass production of *Chaetoceros muelleri* cultured under different environment and culture media. F/2: Guillard f media, T15: triple15 (Nutrimon®, Monomeros, Colombia), H: humus extract (Nutrimon®, Monomeros, Colombia) and K: growth rate values. Values are means ± standard error.
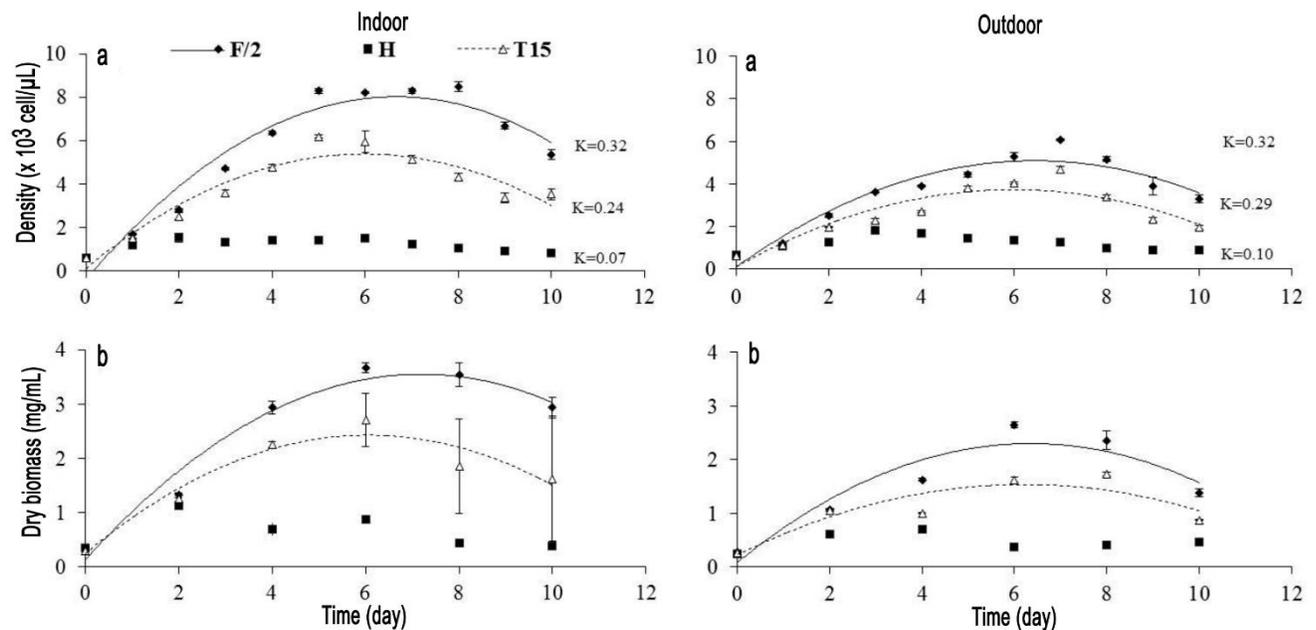
Figure 2. a) Growth in cell density and b) Biomass production of *Chaetoceros calcitrans* cultured under different environment and culture media. F/2: Guillard f media, T15: triple15 (Nutrimon®, Monomeros, Colombia), H: humus extract (Nutrimon®, Monomeros, Colombia) and K: growth rate values. Values are means ± standard error.

DISCUSSION

The higher growth of the local strain *Chaetoceros muelleri* cultured with the F/2 media in comparison with organic fertilizers (T15 and H) agrees with the results obtained in some studies (Newmark *et al*., 1988; Voltolina *et al*., 1998; González *et al*., 1999; Godinez *et al*., 2000; Prieto *et al*., 2005), but contradicts the positive results obtained with of organic fertilizers in other studies (Nieves *et al*., 1996; Valenzuela-Espinoza *et al*., 2002, 2005; Piña *et al*., 2007). The lack of micronutrients and/or the low values of N:P in the orga-
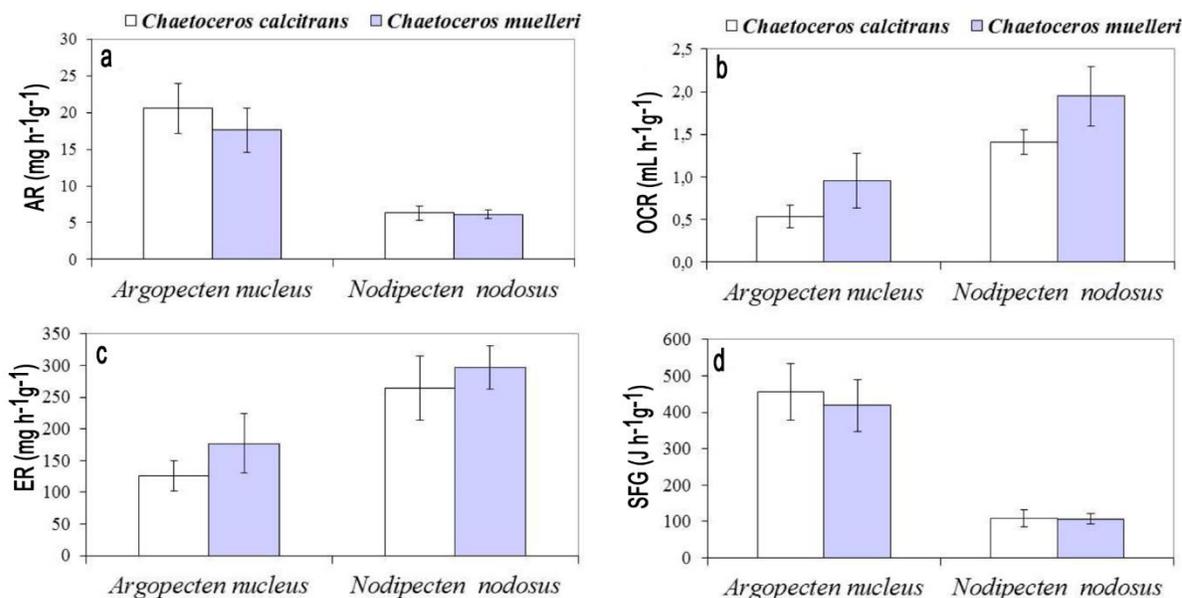
Figure 3. Physiological responses of *Argopecten nucleus* and *Nodipecten nodosus* feed with *Chaetoceros calcitrans* and *Chaetoceros muelleri*. a) Absorption rate, b) oxygen consumption rate, c) ammonium excretion rate, and d) scope for growth. Values are means ± standard error.

nic fertilizers media (Table 1) could limit the photosynthesis and/or growth of both microalgae as have been reported previously in phytoplankton (Menzel & Ryther, 1961) and benthic microalgae (Hillebrand & Soomer, 1999). According to Takeda (1970) and Khoi *et al*. (2006), the optimal N:P value for *Chaetoceros calcitrans* growth is among 12 and 23. In this study, the F/2 treatments had a similar ratio at the beginning of the experiment, but the N:P values of H and T15 treatments were lower (Table 1). These results suggest that the organic fertilizers media tested are inappropriate for the production of the diatoms strains studied.

The lower growth values densities and biomass production of *C. muelleri* under F/2 and outdoor conditions contradicts similar studies results (López-Elías *et al*., 2005; Banerjee *et al*. 2011) and the positive effect of high values of temperature and lighting on the tropical microalgae growth reported before (Rhee & Gotham, 1981; Bermúdez *et al*., 2002; Renaud *et al*., 2002; Velasco *et al*., 2009b; Hemalatha *et al*., 2012). Considering that microalgal growth is affected by light and thermal acclimation (Karsten *et al*., 2006; Staehr & Birkeland, 2006), it is possible that the two generations of previous microalgae exposition to the outdoor conditions were insufficient. So, the higher values of temperature and lighting under outdoor conditions could cause lower uptake of nutrients and/or photo-inhibition as has been previously reported (Durbin, 1974; Vonshak & Guy, 1992; Singh *et al*., 2015), in addition, microalgae cultures under outdoor conditions

are more exposed to bacteria and protozoa contamination (Hu & Gao, 2006; Sandnes *et al*., 2010), increasing the resource competence (Grossart, 1999) and the energetic costs for antimicrobial agents production (Fukami *et al*., 1997, Steinberg *et al*., 1997; Gross, 2003). On the other hand, the low and similar densities and biomass production of *C. muelleri* at indoor and outdoor conditions with organic fertilizers, suggests that under suboptimal media culture conditions, the culture environment is not important. Then, if the use of F/2 media culture and/or indoor facilities is not economically feasible, *C. muelleri* can be produced under outdoor conditions and using T15 media, obtaining around the half of the production showed in the optimal conditions.

The similar or higher cell and biomass production values of *C. muelleri* compared with *C. calcitrans* in this study and with those reported for the species and genera in other studies performed without the injection of CO_2 (Table 3), indicates that the local strain can be produced using the same standard microalgae culture techniques.

Despite the higher protein, lipid and energy content of *C. muelleri* compared to *C. calcitrans*, the physio-logical rates of *Argopecten nucleus* and *Nodipecten nodosus* fed with these two diatoms were not different. Similar results have been reported for the physiological variables and/or growth of bivalves fed with microalgae strains from the same genus such as *Isochrysis* (Ewart & Epifanio, 1981) and *Chaetoceros* (Velasco, 2007) or

geographical origin (Brown *et al.*, 1998). However, other studies have found that growth and/or survival variables of bivalves are higher when fed with some local algae strains such as *Crassostrea gigas* fed with *Pavlova pinguis* (Brown *et al.*, 1998), *Attheya septentrionalis,* and *Entomoneis* cf *punctulata* (Knuckey *et al.*, 2002) isolated in Australia, *Placopecten magellanicus* fed with *Navicula pelliculosa, Chaetoceros septentrionalis* and *Prymnesium* sp. isolated in Canada (Gouda *et al.*, 2006). Those results are apparently related to their higher content of some polyunsaturated fatty acids, proteins and/or carbohydrates. Our results suggest that *C. muelleri* and *C. calcitrans* have similar nutritive values for *A. nucleus* and *N. nodosus* and that the protein, lipid and/or energetic content of these strains did not affect the scallops' physiological performance.

The higher somatic and gonadic growth values reported previously for *A. nucleus* and *N. nodosus* under sea suspended culture in comparison with those maintained in hatchery conditions (Rupp *et al.*, 2005; Velasco & Barros, 2007, 2009) do not appear be related with the presence of local microalgae such as *C. muelleri* in the diet. Other studies that have compared the nutritional value of local and traditional microalgae strains in bivalves have found different results. Brown *et al.* (1997) found that juveniles of *C. gigas* have a higher growth rate when fed with the local microalga *P. pinguis* in comparison with the non-indigenous strain, *Isochrysis* sp., similar results were obtained by Gouda *et al.* (2006) in *P. magellanicus* fed with local strains. On the other hand, Knuckey *et al.* (2002), obtained higher growth of *C. gigas* fed with the non-indigenous *Thalassiosira pseudonana* than when it was fed with the local algae *Attheya septentrionalis* and *Entomoneis* cf *punctulata*. Our results suggest that the nutritive value of the diatoms studied to be used as native scallops food does not be related to their geographical origin.

In conclusion, higher growth and biomass of the local strain *C. muelleri* were obtained using the F/2 media under indoor conditions as well as was verified in the traditional strain *C. calcitrans*. Both microalgae can be produced and used as feed for scallops with similar performance in microalgal growth, biomass production and scallops physiological condition. Then, *C. muelleri* could be produced and used as food for the Caribbean scallops, avoiding the microalgae importation and introduction risk of exotic species into the natural ecosystems, but do not offer any productive advantage.

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