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Research Article

Potential of refrigerated marine cyanobacterium *Synechococcus elongatus* used as food for *Artemia franciscana*

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ABSTRACT. The aim of this study was to evaluate the performance of the cyanobacterium *Synechococcus elongatus* after refrigerated storage (4°C) and examine its potential as food for *Artemia franciscana*. Non-axenic semicontinuous cultures of *S. elongatus* were maintained at 4°C for 8 weeks. In our bioassay, we fed *A. franciscana* with refrigerated *S. elongatus* and fresh cultures of the diatom *Chaetoceros muelleri* as a control. The *S. elongatus* cultures could be refrigerated for up to 8 weeks without loss of its viability or alteration in its growth rate, cell size, and proximate composition. Fresh and eight week-refrigerated cultures of *S. elongatus* were similar with regard to fatty acid profiles. Significant differences in fatty acid profiles were found between refrigerated *S. elongatus* and fresh *C. muelleri* cultures. *Vibrio* bacteria were not detected in any of the *S. elongatus* cultures (fresh or refrigerated) or fresh cultures *C. muelleri*. This work demonstrated that refrigerated *S. elongatus* can be used to feed *A. franciscana*, maintaining similar growth rates and proximate composition compared with fresh *C. muelleri* cultures as feed. Thus, *S. elongatus* has potential aquaculture use as feed for *A. franciscana*.

Keywords: *Synechococcus elongatus*, *Artemia franciscana*, proximate composition, fatty acid, diet, refrigerated storage.

INTRODUCTION

The high costs that are associated with microalgae production, contamination risks, and seasonal variations in the food value of microalgae are significant factors for any aquaculture operation that depends on the mass culture of unicellular algae (Lavens & Sorgeloos, 1996; Meuller-Feuga, 2004). The maintenance of the viability and biochemical composition of microalgae throughout periods of intensive growth is a laborious task because these cultures must be transferred continuously, usually every week (Andersen, 2005). One solution to this problem is to harvest high amounts of microalgae and refrigerate them for a short time to sustain viability and nutritional composition. Refrigerated microalgae can be used to restart new cultures when algal production has decreased (Sánchez-Saavedra, 2006). A wide variety of methods, including storage under oil (Day *et al*., 1997), drum drying (Soeder, 1986; Laing & Millican, 1992), lyophilization (Molina-Grima *et al*., 1994), and storage at low and ultralow temperatures (Ben-Amotz & Gilboa, 1980; Paniagua-Chávez & Voltolina, 1995; Taylor & Fletcher, 1999), have been used for long-term preservation of microalgae.

In recent years, drum-dried and lyophilized microalgae have been introduced to the market for use as food for certain larvae and filter feeders (Richmond, 1986; Andersen, 2005). Cryopreservation of biological specimens has been achieved successfully using protocols that have been developed more or less empirically (Karlson & Toner, 1996). The benefit of this method is that cells can be stored for long periods without lacking viability and changes in their biochemical composition. This approach is used widely in the conservation of genetic resources of many plants and animals. However, for several aquaculture activities, such as growing microalgae for use as food, the cryopreservation of microalgae does not have any significant advantages compared with drum drying and lyophilization. Further, cryoprotectant solutions, when used to enhance the viability of microalgae, might have toxic effects on organisms that consume thawed microalgae, because such substances cannot be eliminated from the culture media (Tzovenis *et al*., 2004).

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Short-term storage at 4°C is an economical and simple method that has been used to store concentrated live microalgae (Chen, 2001; Sánchez-Saavedra, 2006; Sánchez-Saavedra & Núñez-Zarco, 2012, 2015). In the past several years, some companies have introduced several species of microalgae to the market as concentrated pastes that can be maintained at 4°C and used as food. Although the nutritional value of these microalgae is satisfactory, they cannot be used as inocula due to their poor viability (Day *et al*., 1997; Andersen, 2005).

Much effort has been made to preserve the microalgae species that are most commonly used as food in aquaculture (Beaty & Parker, 1992; Cañavate & Lubian, 1994, 1995a, 1995b, 1997; Cañavate & Fernández-Díaz, 2001). However, endemic microalgae cultures could be novel options for feeding local species, because these microalgae are part of their natural diet. For example, *Synechococcus elongatus* was isolated from a shrimp farm in the coastal lagoon of Nayarit, México (21°32'N, 105°17'W) (Aguilar-May, 2002). This cyanobacterium has been considered to have tremendous potential for aquaculture use due to its advantageous properties, such as its high tolerance to variations in light and temperature (Aguilar-May, 2002; Castillo-Barrera *et al.*, 2002), biochemical composition (Campa-Ávila, 2002), ability to remove nutrients (Aguilar-May & Sánchez-Saavedra, 2009; Castro-Ceseña *et al*., 2015), and high food quality for the rotifer *Branchionus plicatilis* (Campa-Ávila, 2002). The diatom *Chaetoceros muelleri* is widely used as food for mollusks and is frequently used to feed *Artemia* in several commercial hatcheries (Gómez-Gil *et al.*, 2002; López-Elías *et al*., 2003, 2005; Meuller-Feuga, 2004). However, *C. muelleri* is not tolerant to extensive changes in temperature and light (Olaizola & Yamamoto, 1994; Brown *et al*., 1997; Piña *et al.*, 2006; Liang *et al*., 2006).

Artemia is a widely applied zooplankton strain for food in aquaculture activities, due to their adaptability to environmental conditions (tolerance to salinity, temperature, and irradiance), feasibility of storing their cysts for several years, ease of hatching of the Artemia nauplii, nutritional value, and low cost (Lavens *et al*., 1989; Lavens & Sorgeloos, 1996, 2000; Villamil *et al*., 2003). Also, Artemia is a common biological model for different pharmacological tests, such as assays for determining the ecotoxicity and bioactivity of medicinal plants (Vanhaecke *et al*., 1981; Krishnaraju *et al*., 2005; Pérez & Lazo, 2010).

We hypothesized that the cyanobacterium *Synechococcus elongatus*, stored for eight weeks in a refrigerator, maintains its nutritional value and viability for aquaculture activities. To increase the use of a local *S. elongatus* strain, we evaluated: 1) the viability of *S. elongatus*, after eight weeks of refrigeration (4°C), 2) changes in the cell size of fresh and refrigerated cultures of *S. elongatus*, 3) the growth rate of recovered cells after refrigeration, 4) weekly changes in the proximate composition of the refrigerated of *S. elongatus*, 5) fatty acid profiles of *S. elongatus* and *C. muelleri*, used as food for *Artemia franciscana*, 6) *Vibrio* loads from fresh and refrigerated cultures of *S. elongatus*, and 7) performance of refrigerated *S. elongatus* as feed for *A. franciscana*.

MATERIALS AND METHODS

Culture conditions of *Synechococcus elongatus*

The strain that we used was the cyanobacterium *Synechococcus elongatus* isolated from Nayarit, México by Aguilar-May (2002). Non-axenic semi-continuous cultures were maintained by 20% daily dilution in 18-L carboys with 10 L "f" medium (Guillard, 1975) and twice the concentration of vitamins as described by Aguilar-May (2002). The culture conditions were: temperature 23 ± 1°C, salinity 33 ± 1, sufficient stirring with air bubbling, and pH between 7.5 and 8.5. The pH was maintained with Tris (hydroxymethyl amino-methane) as described by Guillard (1975). Continuous light (100 µmol m^{-2} s^{-1}) was supplied by two 40 W Philips cool-white fluorescent lamps (1.2 m length).

Culture conditions of *Chaetoceros muelleri*

Fresh cultures of *Chaetoceros muelleri* were used as the control for the feeding bioassays. Cells of *C. muelleri* were obtained from "Centro Reproductor de Especies Marinas (CREMES)" Sonora, México and maintained in triplicate as non-axenic semi-continuous cultures with 30% daily dilution in 18-L carboys (Pacheco-Vega & Sánchez-Saavedra, 2009) with 10 L "f" medium (Guillard, 1975) under similar culture conditions as the *S. elongatus*.

Refrigerated storage of *Synechococcus elongatus* culture

After one week of growth, the *S. elongatus* cultures were considered to be steady (stationary growth phase), and 2 L of the culture was harvested from each carboy, distributed into 24 sterilized, transparent glass bottles (Gerber® flasks) , covered with aluminum foil, and stored at 4°C in a refrigerator for eight weeks.

Evaluation of viability

Viability was measured as the ability of cyano-bacterium to grow after refrigeration. After each week

refrigeration (week 1 to 8), 3 bottles were taken from the refrigerator, from each of which 3 mL aliquots were used as inocula. The inocula were added to 15 mL culture tubes that contained 10 mL "f" medium (Guillard, 1975). The cultures were maintained under the same conditions as described above, excluding the aeration. The tubes were stirred manually for 5 s daily to avoid precipitation of cells. Viability was estimated using daily measurements of the optical density of the tubes at 550 nm with a Hach DR 4000 UV spectrophotometer against a standard curve that had been generated using optical density and cell concentration.

Evaluation of cell size and growth rate

Cell size was determined weekly by measuring the diameter, length, and width of 30 randomly selected cells. Cells were visualized with a compound microscope (Olympus model BH 2) using a digital camera and Image Pro-Discovery, version 5.1. Growth rates were calculated in the exponential growth phase by $\log_2$ transformation (Arredondo-Vega & Voltolina, 2007) and the equation described by Fogg & Thake (1987).

Proximate composition

The proximate cell composition was determined weekly in triplicate sets of fresh cultures (week 0) and refrigerated samples of *S. elongatus*. Aliquots (5 mL) were passed through Whatman GF/C glass filters. Proteins were extracted using 0.1 N NaOH according to the methodology described by Campa-Ávila (2002) and determined according to Lowry *et al*. (1951) using bovine albumin (98%) as the standard. Carbohydrates were extracted following the methodology of Whyte (1987) and the determination was performed according to Dubois *et al.* (1956) using glucose (99%) as the standard. Lipids were extracted following the methodology of Bligh & Dyer (1959) and determined according to the method of Pande *et al.* (1963) using tripalmitin (99%) as the standard.

All amounts in the proximate analysis were expressed as a percentage of the organic fraction of the cells. The organic dry weight was measured by placing the samples in an oven at 60°C, and ash content was generated by incinerating the samples at 470°C according to methodology of Sorokin (1973). The organic dry weight and proximate composition of fresh cultures of *C. muelleri* were also determined as described above.

Fatty acids

Samples (500 mL) of fresh and refrigerated (8 weeks) *S. elongatus* and fresh *C. muelleri* were centrifuged at 4000 rpm for 10 min at 4°C, and the concentrated biomass was maintained individually, stored at -80°C, and lyophilized to determine fatty acid profiles. Total lipids were extracted according to the methodology described by Folch *et al*. (1957). Fatty acid methyl esters (FAMEs) were measured according to Metcalfe *et al*. (1966) and analyzed on a gas chromatograph (GC Agilent Technologies 7890A) using a flame ionization detector and capillary column (Agilent J&W, 123-3232 DB-FFAP, 30-m length, 0.320 mm inner diameter, 0.25 µm film thickness). Hydrogen was used as the carrier gas. The initial temperature was 120°C, which was increased to 230°C in 4 min. FAMEs were identified, based on a comparison of their retention times to those of a commercial standard (37 Component Supelco FAME Mix Sigma). The concentration of each fatty acid was calculated using Chem Station, B.04.01 (Agilent, USA).

***Vibrio* loads**

Concentrations of *Vibrio* spp. in fresh and refrigerated cultures of *S. elongatus* and *C. muelleri* were measured by passing 10 mL of culture through 47 mm MF Millipore (nitrocellulose with 0.22 µm pore aperture) filters. The filters were then incubated with thiosulfate-citrate-bile salts-sucrose agar (TCBS) medium (DIFCO) at 37°C for 24 h. The concentration of *Vibrio* was determined as colony-forming units (CFU) according to Kobayashi *et al*. (1963).

Feeding bioassay

Commercial *Artemia franciscana* cysts were obtained from San Francisco Bay Brand. To obtain organisms with the same age, the covering over the cysts was removed with 6% sodium hypochlorite (Da Rocha *et al*., 2005) and incubated in a 2 L conic container with 1.5 L seawater at 20°C with aeration for 8 h. The nauplii were collected on a 200 µm mesh screen, washed with UV-treated seawater, and distributed to six 500 mL plastic containers to a final concentration of 1 nauplii per milliliter. Nauplii's culture conditions were: temperature 21 ± 1°C; salinity 33 ± 1; stirring with frothing, provided by an aeration stone throughout the entire culture; oxygen concentration 7 ± 0.5 mg L^{-1}; pH between 8.0 and 8.5; continuous light by two 40 W Philips cool-white fluorescent lamps at an irradiance of 50 µmol m^{-2} s^{-1} (1.2 m length); filtered seawater (10, 5, and 1 µm cartridges and activated carbon); disinfection with 6% chlorine (3 mL L^{-1}) for 24 h; and precipitation with sodium thiosulfate (0.15 g L^{-1}).

A set of three separate plastic containers, as described above, selected at random and used to fill another set of 500 mL plastic containers. The final concentration of nauplii in each container was 125 L^{-1}.

Nauplii were fed with refrigerated (8 weeks) *S. elongatus* cells. This set of containers was defined as the experimental group. For the control group, another set of 500 mL plastic containers was prepared as described above, and the nauplii were fed fresh *C. muelleri* cultures. Food ratios were calculated according to Tackaert *et al*. (1987), and increased, depending on the age of *A. franciscana*.

The experiment was completed after 7 days. All treatments were maintained in triplicate. An unfed control group was not included in this experiment because, in previous trials, they survive for only 2 days (data not shown). Each day, 10 *A. franciscana* were collected from each treatment and measured under a light microscope (Olympus model BH-2) with regard to total length. For these measurements, photographs were taken with a digital camera using Image Pro-Discovery, version 5.1. *A. franciscana* survival was determined, based on the initial and final numbers, and expressed as a percentage. At the end of the experiment, the total biomass of *A. franciscana* was harvested and washed with ammonium formate (3%) to remove salt residues; then, the dry weight was estimated by placing the samples in an oven at 60°C as in Sorokin (1973). The proximate composition of *A. franciscana* was also determined as described above.

Data analysis

One-way analysis of variance (ANOVA Sokal & Rohlf, 1979) was used to compare the cell growth, cell size, proximate composition (proteins, carbohydrates, lipids, and ash content), and dry weight of *S. elongatus* at different refrigeration storage times. The same analysis was performed to compare the proximate composition between *S. elongatus* and *C. muelleri*. The fatty acid content of the strains was not compared due to the lack of repeated samples in each treatment. One-way analysis of variance was used to compare the length, survival, and proximate composition of *A. franciscana* that was fed refrigerated *S. elongatus* or fresh *C. muelleri* as a control. Statistical analyses were performed using Statistica 7.0 for Windows® (StatSoft Inc., 1996). A value of $P < 0.05$ was chosen as the level of significance.

RESULTS

Viability of refrigerated *Synechococcus elongatus*

No significant differences in viability ($P = 0.09$) were found between fresh (week 0) and refrigerated *S. elongatus*. The average cell concentration of *S. elongatus* cultures that were established with fresh (week 0) inocula was 31.6×10^6 cell mL^{-1}, *versus* 39.3×10^6 cell mL^{-1} with inocula that had been refrigerated for up to 8 weeks (Fig. 1).

Cell size and growth rate of refrigerated *Synechococcus elongatus*

Cell size did not differ between fresh (week 0, 2.6 ± 0.1 μm) and refrigerated (8 weeks, 2.5 ± 0.2 μm) *S. elongatus* ($P > 0.05$) (Table 1). Also, no significant differences in growth rates ($P > 0.05$) were found between cultures that were started from fresh (week 0) and refrigerated (up to 8 weeks) inoculum. The growth rate of fresh *S. elongatus* cultures (week 0) was 0.30 ± 0.1 divisions d^{-1}, 0.32 ± 0.8 divisions d^{-1} for cultures that were initiated from inoculum that had been refrigerated for 1 week, and 0.35 ± 0.6 divisions d^{-1} for 8-week-refrigerated *S. elongatus* (Table 1).

Proximate composition of refrigerated *Synechococcus elongatus*

The proximate composition (proteins, carbohydrates, and lipids) of *S. elongatus* was similar when maintained fresh (week 0) or refrigerated for up to 8 weeks ($P = 0.089$). The average protein concentration at week 0 was 40.13 ± 0.64% *versus* 42.47 ± 0.52% for refrigerated cultures at the end of the experiment (week 8) (Table 2). The average carbohydrate concentration at week 0 was 23.14 ± 0.82% compared with 22.52 ± 1.19% for refrigerated cultures at week 8 (Table 2). The average lipid concentration at week 0 was 15.25 ± 1.19% and 16.19 ± 0.54% for refrigerated cells at week 8 (Table 2). The ash content of fresh cultures was 27.54 ± 2.74%, compared with 24.61 ± 3.47% at the end of the experiment (week 8) (Table 2).

Fatty acids

The fatty acid content was similar between fresh and refrigerated cultures of *S. elongatus* (Table 3) but differed between fresh and refrigerated cultures of *S. elongatus* and fresh *C. muelleri* cultures (Table 3). Yet, the content of saturated fatty acids was similar between *S. elongatus* and *C. muelleri*. The levels of C16:0 and total saturated fatty acids were slightly higher in *S. elongatus*, whereas C16:1n-7 and total monounsaturated fatty acid levels were higher *C. muelleri*. Polyunsaturated fatty acids and C18:3n-3 content was high in fresh and refrigerated *S. elongatus* compared with *C. muelleri*. However, several polyunsaturated fatty acids (PUFAs), such as ARA, EPA, and DHA, were not detected in *S. elongatus*.

Vibrio loads

No Vibrio bacteria were detected in any *S. elongatus* culture (fresh or refrigerated) or in the fresh *C. muelleri* cultures.

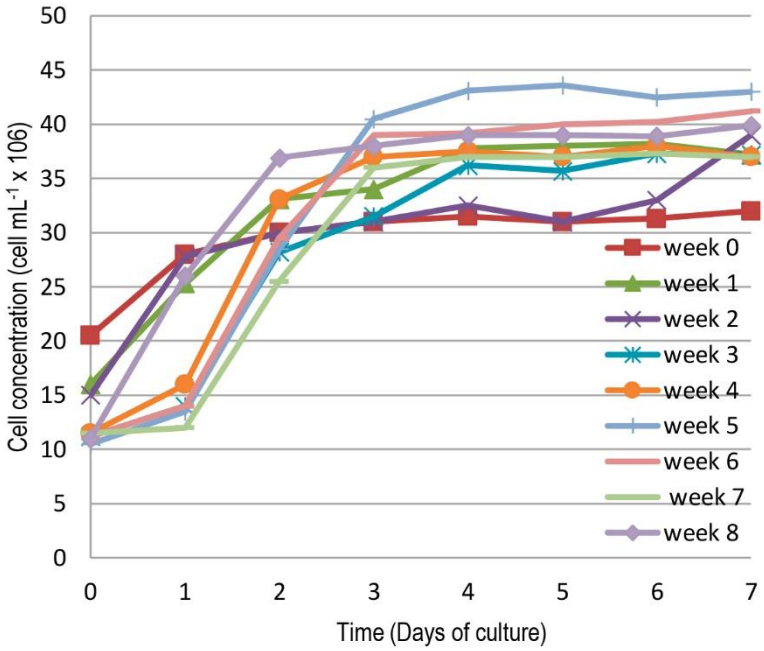

Figure 1. The abundance of cultures started from fresh (week 0) and refrigerated (1 to 8 weeks) inoculum of *Synechococcus elongatus.*

Table 1. Mean values and standard deviation of growth rate and cell size of cultures started from fresh (week 0) and refrigerated (1-8 weeks) inoculum of *Synechococcus elongatus.*

Refrigeration time (weeks)	Growth rate (divisions/day)	Cell size (μm)
0	0.30 ± 0.1	2.6 ± 0.1
1	0.32 ± 0.8	2.6 ± 0.1
2	0.33 ± 0.1	2.6 ± 0.1
3	0.32 ± 0.2	2.6 ± 0.2
4	0.30 ± 0.5	2.6 ± 0.2
5	0.34 ± 0.7	2.5 ± 0.3
6	0.32 ± 0.8	2.6 ± 0.1
7	0.35 ± 0.3	2.5 ± 0.3
8	0.34 ± 0.6	2.5 ± 0.2

Feeding bioassay

The protein and carbohydrate content was significantly higher in *S. elongatus* than in *C. muelleri*. However, *C. muelleri* contained significantly more lipids and ash (Table 4a). The proximate composition of A. franciscana adults did not differ significantly ($P > 0.05$) when fed refrigerated *S. elongatus* cells or fresh *C. muelleri* cultures (Table 4b).

The length of *A. franciscana* nauplii was similar ($P > 0.05$), regardless of being fed refrigerated S. elongatus cells or fresh *C. muelleri* cultures (Table 5). The length of *A. franciscana* metanauplii, juveniles, and adults was significantly higher ($P < 0.05$) when fed refrigerated *S. elongatus*. The survival of *Artemia* was similar when fed refrigerated *S. elongatus* or fresh *C. muelleri*; however, the weight of *A. franciscana* adults of was significantly higher ($P < 0.05$) when given refrigerated *S. elongatus* as food.

DISCUSSION

Cyanobacteria are usually cosmopolitan organisms with a simple cellular organization and high adaptive plasticity, which are beneficial for use in aquaculture. Certain cyanobacteria tolerate high and low temperatures and can grow in darkness and use atmospheric nitrogen (Taylor, 1981). However, several factors can affect the recovery of cyanobacteria when they are exposed to extreme conditions, the most significant of which are the growth cycle from which they were harvested, growth stage, cell density, cell size, and culture conditions (Ben-Amotz & Gilboa, 1980).

Cyanobacteria are found in hot springs and thermal pools and are the dominant organism in Polar lakes (Stal, 1995; Graham & Wilcox, 2000). Based on strains that have been isolated from polar lakes, the temperature for the growth of most cyanobacteria ranges from 15 to -35°C. This finding suggests that such strains are not specifically adapted to low temperatures but can tolerate suboptimal temperatures.

Table 2. Mean values and standard deviation of percentages of proximate composition from fresh (week 0) and refrigerated (1-8 weeks) *Synechococcus elongatus.*

Refrigeration time (weeks)	Proteins (%)	Carbohydrates (%)	Lipids (%)	Ash (%)
0	40.13 ± 0.64	23.14 ± 0.82	15.25 ± 1.19	27.54 ± 2.74
1	41.17 ± 0.85	22.64 ± 1.55	14.26 ± 0.79	24.96 ± 2.34
2	41.85 ± 0.52	23.22 ± 1.18	15.22 ± 0.68	24.24 ± 2.55
3	42.14 ± 0.29	22.59 ± 1.51	14.92 ± 0.87	22.96 ± 3.28
4	41.15 ± 0.96	23.54 ± 1.80	16.05 ± 0.75	25.62 ± 2.37
5	42.55 ± 0.92	22.63 ± 1.15	15.72 ± 0.84	25.54 ± 2.22
6	40.85 ± 0.83	22.58 ± 1.13	14.62 ± 0.66	26.84 ± 2.81
7	42.47 ± 0.52	22.99 ± 1.45	14.53 ± 0.39	29.67 ± 2.30
8	42.15 ± 0.89	22.52 ± 1.19	16.19 ± 0.54	24.61 ± 3.47

Table 3. Percentage of fatty acids (from dry weight) of fresh *Synechococcus elongatus* and after 8 weeks of refrigerated storage and *Chaetoceros muelleri* (fresh culture used as a control without refrigeration).

Component	*Synechococcus elongatus* 8 weeks refrigerated	*Synechococcus elongatus* fresh cultures	*Chaetoceros muelleri* fresh cultures
Saturated			
C4:0	0.17	0.16	-
C6:0	0.02	0.02	-
C8:0	0.01	0.01	-
C10:0	0.01	0.01	-
C13:0	0.04	0.05	0.02
C14:0	0.54	0.56	9.99
C15:0	0.40	0.40	0.64
C16:0	39.80	39.96	28.44
C17:0	1.10	1.12	0.09
C18:0	2.48	2.36	1.94
C22:0			0.47
Total	44.58	44.65	41.58
Monounsaturated			
C14:1n-5	0.13	0.12	0.07
C15:1n-5	0.07	0.06	0.05
C16:1n-7	16.56	16.33	39.02
C17:1n-7	2.21	2.31	0.07
C18:1n-9	8.75	8.37	1.23
Total	27.72	27.19	40.43
Polyunsaturated			
C18:2n-6	9.90	10.25	0.69
C18:3n-6	0.03	0.08	2.63
C18:3n-3	17.76	17.83	0.24
C18:2n-6			0.19
C20:2n-6			0.65
C20:3n-3			0.21
C20:4n-6 (ARA)			0.04
C20:5n-3 (EPA)			12.18
C22:6n-3 (DHA)			1.17
Total PUFA	27.70	28.16	17.98
Total n-3 PUFA	17.76	17.83	13.80
Total n-6 PUFA	9.93	10.33	4.20
Grand total	100.00	100.00	100.00

Table 4. Mean values and standard deviation of proximal composition (percentage of dry weight) of *Synechococcus elongatus* after 8 weeks of refrigerated storage, *Chaetoceros muelleri* (fresh culture used as control without refrigeration) (A), and *Artemia franciscana* adults fed *S. elongatus* after 8 weeks of refrigerated storage and *C. muelleri* (fresh culture used as control without refrigeration) (B). Equal letters indicate lack of significant differences by one-way ANOVA and Tukey *a posteriori* test $\alpha = 0.05$: a > b.

a	*Synechococcus elongatus*	*Chaetoceros muelleri*
Proteins	42.15 ± 0.89a	34.81 ± 3.52b
Carbohydrates	22.52 ± 1.19a	13.80 ± 2.75b
Lipids	16.19 ± 0.54a	17.23 ± 2.57a
Ash	24.61 ± 3.47b	36.92 ± 1.32a
b	*Artemia* adults fed with *Synechococcus elongatus*	*Artemia* adults fed with *Chaetoceros muelleri*
Proteins	34.61 ± 2.23a	35.87 ± 1.99a
Carbohydrates	21.36 ± 1.59a	18.83 ± 2.85a
Lipids	17.19 ± 4.43a	18.28 ± 4.87a
Ash	27.29 ± 3.94a	29.76 ± 2.89a

Table 5. Mean values and standard deviation of total length, dry weight, and survival of different stages of *Artemia franciscana* fed *Synechococcus elongatus* after 8 weeks of refrigerated storage and *Chaetoceros muelleri* (fresh culture used as a control without refrigeration). Equal letters indicate lack of significant differences by one-way ANOVA and Tukey *a posteriori* test $\alpha = 0.05$: a>b. NE: sample not measured.

	Nauplii		Metanauplii		Juveniles		Adults	
	Fed with refrigerated *S. elongatus*	Fed with fresh *C. muelleri*	Fed with refrigerated *S. elongatus*	Fed with fresh *C. muelleri*	Fed with refrigerated *S. elongatus*	Fed with fresh *C. muelleri*	Fed with refrigerated *S. elongatus*	Fed with fresh *C. muelleri*
Survival (%)	100	100	100	100	99	98	99	97
Length (mm)	730.74 ± 11.3a	732.84 ± 12.41a	1822.82 ± 54.12a	1716.93 ± 43.12b	4233.86 ± 47.73a	4098.19 ± 54.33b	5698.54 ± 43.54a	4875.29 ± 44.76b
Weight (mg)	NE	NE	NE	NE	NE	NE	27.89 ± 2.17a	23.22 ± 1.53b

Resistance to desiccation, freeze-thaw cycles, and high solar irradiation and their maintenance of a slow growth rate contribute to their survival in polar aquatic systems (Tang *et al*., 1997). Thus, to optimize their recovery, we established the ideal conditions for *S. elongatus* and refrigerated this cyanobacterium when the cultures were in the exponential phase of growth to obtain the peak cell densities and high protein content (Fogg & Thake, 1987).

Our results showed the high adaptability of *S. elongatus* to extreme changes in temperature and light. The significant viability of refrigerated *S. elongatus* under darkness may be attributed to their ability to slow their metabolism. The growth rates of fresh and refrigerated *S. elongatus* were low (~0.30 divisions d^{-1}) compared with ~0.48 divisions d^{-1}, as reported by Aguilar-May (2002) and Campa-Ávila (2002) for the same strain. These differences could be due to the high culture volume and lower light intensity used in the experiments described by the latter authors.

The nutritional value of microalgae must be considered because the performance of organisms that consume it depends on its nutritional profile (Richmond, 2003; Muller-Feuga, 2004). Although many species of microalgae that are suited for mass culture are used as food in aquaculture, not all yield good growth and survival, due to differences in biochemical composition and environmental adapta-bility (Webb & Chu, 1983; Enright *et al*., 1986; D'Souza & Loneragan, 1999). We did not detect any differences in biochemical composition between fresh and refrigerated cultures, indicating that *S. elongatus* decreased its metabolic activity and did not use storage products. Live microalgae cells have several strategies for surviving at low temperatures, such as the production of antifreeze proteins, accumulation of polyunsaturated fatty acid chains, or over-excitation of photosynthetic apparatus (Morgan-Kiss *et al.*, 2006).

The nutritional value of refrigerated *S. elongatus* was good when used as feed for *A. franciscana*. The strain *C. muelleri* was used as a control because this diatom is widely used in shrimp farms in northwest México (López-Elías *et al*., 2005). The similar proximate composition of *A. franciscana* adults that were fed refrigerated *S. elongatus* or fresh *C. muelleri*

cultures demonstrated that refrigerated *S. elongatus* meets the nutritional requirements of *A. franciscana*.

The fatty acid profile of *S. elongatus* and *C. muelleri* differed, and their compositions were what has typically been reported for cyanobacteria and diatoms (Sánchez-Saavedra & Voltolina, 1995; Campa-Ávila, 2002; Renaud *et al*., 2002). Cyanobacteria usually harbor high content of C16:1 and C18:1 and low polyunsaturated fatty acids (Anahas & Muralitharan, 2015). Conversely, diatoms have high concentrations of 14:0, 16:0, 16:1(n-7), and 20:5(n-3), constituting 70% to 90% of the total fatty acid content (Volkman *et al*., 1989; Brown *et al*., 1997; Strandberg *et al.,* 2015). In our study, for *C. muelleri*, these fatty acids represented over 90% of total fatty acids, *versus* 56.86% to 56.90% for fresh and 8-week-stored *S. elongatus*. Thus, the proximate composition of *A. franciscana* adults could be attributed to the findings that it is not extrinsically determined in the long term and that *A. franciscana* can modify its dietary amino and fatty acid levels (Lavens *et al*., 1989; Sánchez-Saavedra & Voltolina, 1995).

The growth of *A. franciscana* nauplii did not differ when fed *C. muelleri* or *S. elongatus*, possibly due to storage reserves in the nauplii. However, metanauplii that were fed refrigerated *S. elongatus* were larger than those that were given *C. muelleri*. Overall, the length of *A. franciscana* at different stages under both experimental culture conditions was similar to those reported by others authors (Sick, 1976; Correa-Sandoval, 1991).

Infections that are caused by luminescent vibrio can yield dramatic losses in intensive rearing of mollusks, finfish, and lobsters. Cysts of *A. franciscana*, microalgae, and water are the most common vectors with regard to the propagation of *Vibrio* spp. in aquaculture activities (López-Torres *et al*., 2000). We did not detect *Vibrio* in our fresh or refrigerated cultures, perhaps due to the high quality of the cultures and the antibacterial characteristics of *S. elongatus* and *C. muelleri* (Sánchez-Saavedra *et al*., 2010). Consequently, *S. elongatus* can provide additional benefits by reducing bacterial load (González-Davis *et al.*, 2012; Molina-Cárdenas *et al.*, 2014).

Hatchery rearing of bivalve mollusks, certain marine finfish in the early stages, and crustaceans depend on the production of live microalgae. The costs for producing microalgae constitute ~50% of the entire hatchery production (Richmond, 2003). The use of refrigerated microalgae is a viable approach toward maintaining small cultures when there are bottlenecks in the production of microalgae. Further, *S. elongatus* can be used as a biofilter for "green water culture" due to its high capacity for removing nutrients, especially nitrogen and phosphorous (Aguilar-May & Sánchez-Saavedra, 2009; Castro-Ceseña *et al*., 2015).

In conclusion, refrigerated storage of *S. elongatus* renders the cells dormant for up to 8 weeks. This strain has potential use aquaculture, based on its high-quality proximate composition and nutritional value for *A. franciscana*.

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