Latin American Journal of Aquatic Research
E-ISSN: 0718-560X
lajar@ucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

García, Norma; López-Elías, José Antonio; Miranda, Anselmo; Martínez-Porchas, Marcel; Huerta, Nolberta; García, Antonio

Effect of salinity on growth and chemical composition of the diatom Thalassiosira weissflogii at three culture phases

Latin American Journal of Aquatic Research, vol. 40, núm. 2, julio, 2012, pp. 435-440
Pontificia Universidad Católica de Valparaíso
Valparaiso, Chile

Lat. Am. J. Aquat. Res., 40(2): 435-440, 2012
DOI: 10.3856/vol40-issue2-fulltext-18

Research Article

Effect of salinity on growth and chemical composition of the diatom *Thalassiosira weissflogii* at three culture phases

Norma García[1], José Antonio López-Elías[1], Anselmo Miranda[2], Marcel Martínez-Porchas[3] Nolberta Huerta[1] & Antonio García[3]
[1]Departamento de Investigaciones Científicas y Tecnológicas, Universidad de Sonora
Blvd. Luis Donaldo Colosio, Hermosillo, Sonora, 83000, México
[2]Centro de Estudios Superiores del Estado de Sonora, Carretera a Huatabampo
y Periférico Sur Navojoa, Sonora, 85800, México
[3]Centro de Investigación en Alimentación y Desarrollo, La Victoria, Hermosillo
Sonora, 83304, México

ABSTRACT. The estuarine diatom *Thalassiosira weissflogii* (Fryxell & Hasle, 1977) has been widely used as live feed in aquaculture. The growth rate and biochemical composition of microalgae are highly influenced by environmental factors such as, light, salinity and nutrient availability. Salinity is difficult to control in some shrimp laboratories specialized in larvae production, because these laboratories depend upon the levels measured in estuaries or coastal lagoons, which are the water sources for larvae culture. The present study evaluated the effect of different salinities (25, 30, 35, 40, 45 and 50 psu), on the growth and chemical composition of *T. weisflogii* at three culture phases, under laboratory conditions. The highest growth rate and maximum cell density were found at 25 psu. Decrease in size and striking changes in morphology of the cells were observed at the higher salinities and drastic changes occurred at 50 psu. Protein and carbohydrate content were higher at low salinities (25 and 30 psu) during the stationary phase. The lipid production was higher at low salinities, but diminished as the phase changes occurred; in contrast, the lipid content was unaffected by the growth phase at higher salinities (≥35 psu). The higher growth rate and better biochemical composition were obtained at 25 and 30 psu.

Keywords: estuarine diatom, microalgae culture, proximate composition.

Efecto de la salinidad en el crecimiento y composición química de la diatomea *Thalassiosira weissflogii* en tres fases de cultivo

RESUMEN. La diatomea estuarina *Thalassiosira weissflogii* (Fryxell & Hasle, 1977) ha sido utilizada como alimento vivo en acuacultura. La composición bioquímica del alimento vivo afecta la nutrición de los organismos durante su cultivo. La tasa de crecimiento y composición bioquímica de las microalgas están altamente influenciadas por factores ambientales como luz, salinidad y disponibilidad de nutrientes. En algunos laboratorios productores de larvas de camarón, es difícil controlar la salinidad, debido a que éstos dependen de los niveles presentes en estuarios o lagunas costeras, los cuales son la fuente de agua para el cultivo larvario. El presente estudio evaluó el efecto de diferentes salinidades (25, 30, 35, 40, 45 y 50 psu), sobre el crecimiento y la composición proximal de *T. weissflogii* en tres fases de cultivo, bajo condiciones de laboratorio. Las mayores tasas de crecimiento y la máxima densidad celular se obtuvieron a 25 psu. Se observó una reducción en tamaño y cambios en la morfología de las células a altas salinidades y los cambios drásticos ocurrieron a 50 psu. El contenido de proteínas y de carbohidratos fue más elevado a salinidades bajas (25 y 30 psu), durante la fase estacionaria de crecimiento. La producción de lípidos fue elevada a bajas salinidades y disminuyó a medida que cambiaba de fase; no se observó un efecto de las fases del cultivo sobre el contenido de lípidos en altas salinidades (≥35 psu). La mayor tasa de crecimiento y la mejor composición bioquímica se obtuvieron 25 y 30 psu.

Palabras clave: diatomea marina, cultivo de microalgas, composición proximal.

Corresponding author: José Antonio López-Elías (jalopez@guayacan.uson.mx)

INTRODUCTION

Microalgae are the major food source for many aquatic organisms and the main live feed used in marine hatchery operations. The value of microalgae as food source depends on some characteristics such as cell size and biochemical composition (Becker, 2004). In particular, the diatom *Thalassiosira weissflogii* (Fryxell & Hasle, 1977) has been widely used as live feed in aquaculture; however information related to its chemical composition is still scarce (Li, 1979; Emmerson, 1980; Fistarol *et al.*, 2005).

Environmental factors have been reported to influence the chemical composition of microalgae. For instance, the effect of light, nutrients, temperature, pH and salinity has been well documented (Richmond 1986; Henley *et al.*, 2002). In addition, the growth phase has been reported as an intrinsic factor influencing the growth rate and biochemical composition of microalgae (Renaud *et al.*, 2002). Additionally, salinity is one of the most important factors affecting the growth and productivity of plants and algae (Parida & Das, 2005). Salinity affects growth and chemical composition of *T. weissflogii* (Vrieling *et al.*, 2007); therefore, considering that mass cultures are performed outdoor at different ages and salinities (Becerra-Dorame *et al.*, 2010), it is important to evaluate the combination of both variables. Information related to the effect of salinity on the growth and chemical composition of *T. weissflogii* is not yet available. Thus, it is important to know the optimal salinity at which *T. weissflogii* has to be cultured for aquacultural purposes.

In addition, some shrimp and mollusks nurseries use estuaries and coastal lagoons as water sources, but the processes of evaporation or precipitation modify the salinity levels of these water-bodies; as consequence, this phenomenon could affect the quality of microalgae used as source of food in aquaculture. The aim of this study was to determine the effect of different salinities on the growth and chemical composition of the marine diatom *T. weissflogii* at three different phases of growth in batch cultures.

MATERIALS AND METHODS

Strains of *Thalassiosira weissflogii* used in this study were obtained from the algal stock collection of Department of Scientific and Technological Research (DICTUS), México, and maintained under laboratory conditions. The microalgae were cultured under laboratory conditions using 500 mL flasks. The measurements of growth and proximate composition were made at three growth phases: exponential (3^{rd} day), late exponential (5^{th} day) and stationary (7^{th} day).

The culture media used was f/2 (Guillard, 1975). The salinity concentrations used were 25, 30, 35, 40, 45 and 50 psu). Each treatment was performed by triplicate. Room temperature was maintained at 20 ± 1°C with continuous light at an irradiance of 500 mmol m^{-2} s^{-1} (Extech instrument). The pH was measured daily with a pHmeter (pHep, Hanna Instruments).

Cell counts were performed daily, by triplicate, with a Neubauer chamber (0.1 mm depth) to determine the maximum cell density and specific growth rate (μ) day^{-1}, which was calculated by linear regression of the log2 of cell concentration (B) on time (t) at the exponential growth phase: $\mu = (Log_2B_n - Log_2B_o)/(t_n-t_o)$. The cell volume was calculated like cylinder volume using the cell diameters (width) and lengths cell as reported by Roubeix & Lancelot (2008).

For biomass determination (dry weight), 100 mL of culture samples (n = 6) were filtered through pre-weighted 47 mm membrane filters (Whatman). Filtered cells were washed with 25 mL of ammonium formiate (3%), to remove salt precipitates, and dried at 60°C for 8 h to achieve a constant weight in a convection stove. Afterwards, samples were burned in a muffle furnace at 480°C for 12 h and weighted in an analytical balance to obtain the mineral fraction (ashes).

During the exponential, late exponential and stationary growth phases, 70 mL of culture samples (n = 6) were filtered through pre-weighed 47 mm membrane filters (Whatman) for lipid analysis and 15 mL were filtered through 25 mm membrane filters (Whatman) for protein and carbohydrate analyses. Filtered cells were washed with 25 mL of ammonium formiate (3%), to remove salt precipitates. The filters with algae samples were stored at -20ºC for posterior chemical analysis. Total lipid content was extracted according to Bligh & Dyer (1959), and quantifications were made following the methodology described by Nieves *et al*. (2009). Total protein content was determined by method described by Lopez-Elías *et al*., (2005), and carbohydrates were determined according to Sánchez-Saavedra & Voltolina (2006).

Statistical analysis included one-way analysis of variance (ANOVA) with significance level of $\alpha = 0.05$ (Zar, 1996), for maximum cell density, specific growth rate and cell volume, and two-way analysis of variance, with significance level $\alpha = 0.05$, and Duncan multiple comparison tests for biomass and cell biochemical composition.

RESULTS

The maximum cell densities were observed between 25-35 psu (Fig. 1). The maximum cell density (44 10^4 cells mL^{-1}) was obtained at 35 psu, followed by 25 and 30 psu with 43 and 42 x 10^4 cells mL^{-1} respectively. In cultures exposed to higher salinities (40, 45 and 50 psu) the cell density decreased significantly ($P < 0.001$; <41 cells mL^{-1}) (Table 1).

The maximum growth rate was achieved at 25 psu (1.24 day^{-1}) and the lowest were registered at 45 and 50 psu (≥0.8 day^{-1}). Regarding to cell volume, an inverse relation with salinity level was detected, being around 563.7 μm^3 for the microalgae cultured at 50 psu and 1,594.3 μm^3 for 25 psu (Table I).

The biomass production was significantly lower ($P < 0.001$) at salinities of 25 and 30 psu compared to 35 to 50 psu. Significant differences were observed during the culture phases; the major dry biomass was observed at 25-30 psu of the stationary phase (Table 2). In general, the maximum biomass was obtained at late and stationary phases.

Regarding to chemical composition, the protein production was affected by the growth phase in all salinity levels evaluated; significant differences ($P < 0.05$) were observed among growth phases (Table 2). The lowest values corresponded to the exponential phase, followed by the late exponential and the stationary phases respectively. Also, the maximum protein concentrations (350 and 320 mg g^{-1}) were detected at lower salinities (25 and 30 psu respectively). Results show a clear tendency in which the increase of salinity caused a decrease in protein content ($P < 0.001$).

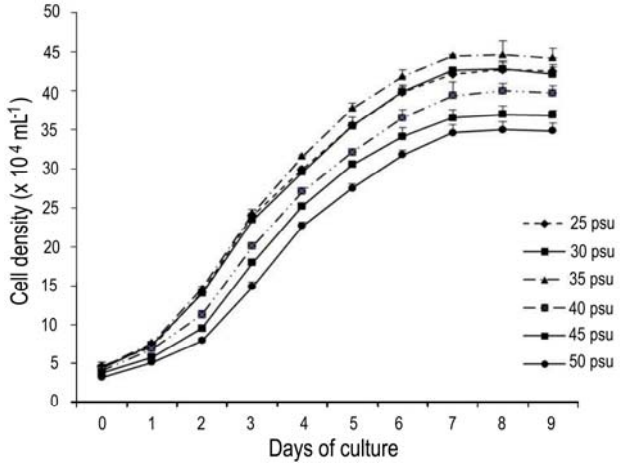

Figure 1. Growth (x10^4 cell mL^{-1}) of the estuarine diatom *Thalassiosira weissflogii* at different salinities. Horizontal bars indicate ± standard error (n = 3).

Figura 1. Crecimiento (x10^4 cel mL^{-1}) de la diatomea marina *Thalassiosira weissflogii* a diferentes salinidades. Las barras horizontales indican ± error estándar (n = 3).

Total carbohydrate production showed a similar tendency than protein content during the growth phases; the lower levels were obtained during the exponential phase and the maximum during the stationary phase (Table. 2).

Unlike the results of proteins and carbohydrates, the lipid production was not affected by the growth phase at 40, 45 or 50 psu (Table 2). On the other hand, significant differences were observed in lipid concentration at the different growth phases in treatments of 25, 30 or 35 psu; the highest lipid concentrations (≈250 mg g^{-1}) were found in microalgae at the exponential phase. However, the lipid concentration diminished in the same treatments (25, 23 and 35 psu) with the increase in the age of the culture (Table 2).

DISCUSSION

Differences in cell density were observed among treatments during the nine days of culture; as consequence, the specific growth rate was significantly higher at lower salinities, because *T. weissflogii* is an estuarine microalgae (Radchenko & Il'yash, 2006). Vrieling *et al.* (2007) found a similar growth response in cultures of two marine diatoms (*T. punctigera* and *T. weissflogii*) at low salinities (25 and 33 psu).

The biomass peak was obtained at higher salinities, possibly due to the increase of the mineral fraction. The increase of the mineral fraction could be a cellular response related to osmotic pressure adjustments (Mansour & Salama, 2004). In this study, the mineral fraction in *T. weissflogii* was greater at salinities higher than 35 psu. For instance, unsatisfactory results have been observed in this case when *T. weissflogii* cultured at >35 psu, are used as feed for shrimp larvae, due to the lower content of organic matter and inadequate profile of macronutrients.

The protein concentrations obtained in this experiment at 25 psu were equal to Kiatmetha *et al.* (2010), who reported a maximum protein value of 326 mg g^{-1} DW at 25 psu in mass cultures of the diatom *T. weissflogii*. The carbohydrate concentration was significantly affected by salinity, the maximum concentrations were detected in late exponential and stationary phases with 25 and 30 psu; these results are consistent with Raghavan *et al*. (2008). Regarding to the effect of salinity, it was observed that the lipid contents decreased as salinity increased.

Table 1. Means and standard deviations of maximum cellular density, specific growth rate and cell volume at different salinities. Different letters show indicate significant differences ($\alpha = 0.05$).

Tabla 1. Medias y desviaciones estándar de la máxima densidad celular, tasa de crecimiento específico y volumen celular a diferentes salinidades. Letras diferentes indican diferencias significativas ($\alpha = 0,05$).

Salinity (psu)	Maximum cellular density ($x10^4$ mL^{-1})	Specific growth rate (μ) day^{-1}	Cell volume ($m\mu^3$)
25	43 ± 0.5^{ab}	1.24 ± 0.025^{a}	1594.3 ± 145.1^{a}
30	42 ± 1.2^{b}	1.12 ± 0.023^{b}	1489.0 ± 151.6^{a}
35	44 ± 1.2^{a}	1.16 ± 0.039^{b}	1401.4 ± 57.2^{a}
40	40 ± 0.9^{c}	0.99 ± 0.030^{c}	1013.9 ± 145.0^{b}
45	37 ± 0.6^{d}	0.82 ± 0.038^{d}	700.0 ± 14.8^{c}
50	35 ± 1.0^{e}	0.81 ± 0.007^{d}	563.7 ± 29.9^{c}

Table 2. Mean and standard deviation of biomass production in dry basis, organic matter and ash in cultures of *T. weissflogii* at six different salinities during three growth phases (exponential, late exponential and stationary).

Tabla 2. Promedio y desviación estándar de la producción de biomasa en base a peso seco, materia orgánica y cenizas en cultivos de *T. weissflogii*, en seis diferentes salinidades durante tres fases de crecimiento (exponencial = exp., lento crecimiento = lento crec. y estacionaria = estac.).

Salinity (psu)	Growth phase	Dry biomass ($g\ L^{-1}$)	Protein ($mg\ g^{-1}$)	Carbohydrates ($mg\ g^{-1}$)	Lipids ($mg\ g^{-1}$)
25	Exponential	0.071^{a} (0.003)	199^{e} (07)	144^{d} (10)	249^{h} (10)
30	Exponential	0.076^{a} (0.005)	177^{d} (17)	152^{de} (10)	247^{h} (16)
35	Exponential	0.092^{b} (0.005)	138^{c} (02)	123^{c} (15)	199^{cd} (08)
40	Exponential	0.108^{c} (0.007)	102^{bc} (04)	87^{a} (04)	169^{ab} (09)
45	Exponential	0.105^{c} (0.004)	99^{ab} (09)	77^{a} (03)	167^{ab} (05)
50	Exponential	0.107^{c} (0.002)	85^{a} (10)	71^{a} (02)	159^{a} (03)
25	Late exp.	0.096^{b} (0.007)	238^{g} (21)	235^{g} (07)	225^{fg} (18)
30	Late exp.	0.090^{b} (0.006)	226^{fg} (10)	240^{gh} (17)	237^{gh} (15)
35	Late exp.	0.132^{e} (0.002)	144^{c} (06)	167^{e} (05)	185^{bc} (06)
40	Late exp.	0.123^{d} (0.004)	140^{c} (10)	166^{e} (03)	169^{ab} (04)
45	Late exp.	0.120^{d} (0.006)	131^{c} (01)	158^{de} (13)	168^{ef} (08)
50	Late exp.	0.126^{de} (0.001)	109^{b} (07)	107^{b} (05)	159^{a} (02)
25	Stationary	0.108^{c} (0.002)	352^{k} (14)	256^{h} (08)	219^{ef} (08)
30	Stationary	0.112^{c} (0.005)	325^{i} (11)	248^{gh} (11)	214^{def} (14)
35	Stationary	0.122^{d} (0.007)	289^{i} (17)	207^{f} (12)	204^{de} (10)
40	Stationary	0.124^{de} (0.003)	269^{h} (12)	200^{f} (10)	176^{ab} (04)
45	Stationary	0.124^{de} (0.004)	235^{g} (02)	206^{f} (07)	169^{ab} (04)
50	Stationary	0.124^{de} (0.006)	212^{ef} (18)	209^{f} (12)	162^{a} (09)

Although many species of microalgae are tolerant to great variations of salinity, their chemical composition can be affected by such factor (Brown *et al.*, 1996) as has been demonstrated in this experiment. Protein, lipid and carbohydrate contents are commonly affected by low or high salinity levels for most microalgae species (Richmond, 1986).

Despite this euryhaline species can thrive at extreme salinities, its nutritional quality can be affected and traduced in poor growth performance for shrimp larvae, considering that much energy and protein are required for metamorphosis.

Microalgae biomass and chemical composition can vary according to the environmental conditions and the age of the culture (Becker, 2004). In this study, it was observed that salinity and growth phase were the main factors influencing the proximal composition of *T. weissflogii.* This information can be useful to produce cells with pre-determinate composition, depending upon the nutritional requirements of particular aquacultural species. It is concluded that salinity fluctuations induce significant changes in the growth rate, biomass production and biochemical composition of *T. weissflogii.* Cell volume, carbohydrate, lipid and protein contents were higher at low salinities, whereas high salinities negatively affected the growth rate, cell volume and organic composition.

REFERENCES

Becerra-Dorame, M., J.A. López-Elías & L.R. Martínez-Córdova. 2010. An alternative outdoor production system for the microalgae *Chaetoceros muelleri* and *Dunaliella* sp. during winter and spring in Northwest Mexico. Aquacult. Eng., 43: 24-28.

Becker, W. 2004. Microalgae for aquaculture: the nutritional value of microalgae for aquaculture. In: A. Richmond (ed.). Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Iowa, pp. 380-391.

Bligh, E.G. & W.J. Dyer. 1959. A rapid method of total lipid extractionand purification. Can. J. Biochem. Physiol., 37: 911-917.

Brown, R.M., A.G. Dunstan, S.A. Norwood & A.K. Miller. 1996. Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. J. Phycol., 32: 64-73.

Emmerson, W.D. 1980. Ingestion, growth and development of *Penaeus indicus* larvae as a function of *Thalassiosira weissflogii* cell concentration. Mar. Biol., 58: 65-73.

Fistarol, G.O., C. Legrand & E. Granéli. 2005. Allelopathic effect on a nutrient-limited phytoplankton species. Aquat. Microb. Ecol., 41: 153-161.

Guillard, R.R.L. 1975. Cultures of phytoplankton for feeding marine invertebrates. In: W.L. Smith & M.M. Chalg (eds.). Culture of marine invertebrate animals. Plenum Press, New York, pp. 29-60.

Henley, J.W., M.K. Major & L.J. Hironaka. 2002. Response to salinity and heat stress in two halotolerant Chlorophyte algae. J. Phycol., 38: 757-766.

Kiatmetha, P., W. Siangdang, B. Bunnag, S. Senapin & B. Withyachumnarnkul. 2010. Enhancement of survival and metamorphosis rates of *Penaeus monodon* larvae by feeding with the diatom *Thalassiosira weissflogii*. Aquacult. Int. DOI 10.1007/s 10499-010-9375-y.

Li, W.K.W. 1979. Cellular composition and physiological characteristics of the diatom *Thala-ssiosira weissflogii* adapted to cadmium stress. Mar. Biol., 55: 171-180.

López Elías, J.A., D. Voltolina, I.S. Avila Mercado, M. Nieves, M. & B., Cordero Esquivel. 2005. Growth, composition and biomasa yields of *Chaetoceros muelleri* mass cultures with different routines and tank depths. Rev. Invest. Mar., 26(1): 67-72.

Mansour, M.M.F. & K.H.A. Salama. 2004. Cellular basis of salinity tolerance in plants. Environ. Exp. Bot., 52: 113-22.

Nieves, M., D.J. López, M.A. Medina, P. Piña, S. Leal & J.A. López-Elías. 2009. Producción y calidad de *Chaetoceros muelleri* a diferentes concentraciones de nutrientes y densidades de inóculo. Rev. Invest. Mar., 30(2): 123-133

Parida, A.K. & A. Das. 2005. Salt tolerance and salinity effects on plants: a review. Ecotox. Environ. Safe., 60: 324-349.

Radchenko, I.G. & L.V. Il'yash. 2006. Growth and photosynthetic activity of diatom *Thalassiosira weissflogii* at decreasing salinity. Plant Physiol., 33(3): 242-247.

Raghavan, G., C.K. Haridev & C.P. Gopinathan. 2008. Growth and proximate composition of the *Chaetoceros calcitrans* f. *pumilus* under different temperature, salinity and carbon dioxide levels. Aquacul. Res., 39: 1053-1058.

Renaud, S.M., L.V. Thinh, G. Lambridis & D.L. Parry. 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture, 211: 195-214.

Richmond, A. 1986. Cell response to environmental factors. In: A. Richmond (ed.). Handbook of microalgal mass culture. CRC Press, Boca Raton, pp. 69-99.

Roubeix, V. & C. Lancelot. 2008. Effect of salinity on growth, cell size and silification of an euryhaline freshwater diatom: *Cyclotella meneghiniana* Kütz. Trans. Waters Bull., 1: 31-38.

Sánchez-Saavedra, M.P. & D. Voltolina. 2006. The growth rate, biomass production and composition of *Chaetoceros* sp. grown with different light sources. Aquacult. Eng., 35: 161-165.

Received: 27 October 2011; Accepted: 13 June 2012

Vrieling, E.G., Q. Sun, M. Tian, P.J. Kooyman, W.W.C. Gieske, R.A. van Santen & N.A.J.M. Sommerdijk. 2007. Salinity-dependent diatom biosilicification implies an important role of external ionic strength. PNAS, 104(25): 10441-10446.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, New Jersey, 920 pp.