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Growth and proximate composition of the *Chaetoceros calcitrans* f. *pumilus* under different temperature, salinity and carbon dioxide levels

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Abstract

The marine diatom *Chaetoceros calcitrans* f. *pumilus* has been examined for its potential source as live feed in aquaculture. The present study investigated effects of temperature (20, 25 and 30 °C), salinity (25 and 35) and carbon dioxide addition (air+CO_2) on the growth and proximate composition of *C. calcitrans* under laboratory conditions. The growth and biomass of *C. calcitrans* were primarily affected by carbon dioxide addition, and to a lesser extent by temperature and salinity. In general, lipid and carbohydrate contents were higher at lower temperatures (20 and 25 °C), while the protein content was unaffected. Carbon dioxide addition increased protein, while lowering carbohydrates, but had no effect on lipid content. Carbohydrates were increased while lipids and protein decreased at the highest salinity (35 ± 0.9). These results should be taken into consideration when evaluating the dietary value of this micro alga for aquaculture.

Keywords: *Chaetoceros calcitrans*, growth, composition, temperature, salinity, carbon dioxide, live feed

Introduction

Micro algae are the major food source for many aquatic organisms and the main live feed component in marine hatchery operations because they serve as a natural resource for polyunsaturated fatty acids. The diatom *Chaetoceros calcitrans* is considered as the most popular strain used in hatcheries, especially for shrimp larvae. This species gives vital energy and organic nutrients for the growth and development of larvae and juveniles (Jeffrey, Brown & Garland 1994). Its culture is an important activity, which influences the nutritional value of aquatic herbivores (Whyte, Bourne & Hodgson 1989) as well as the economic aspects of their culture (Coutteau & Sorgeloos 1992). Very few investigations on the optimal condition for the growth of *C. calcitrans* are available. Liang (1985) reported the effect of silicate and its optimum level on growth of this diatom. The operation of a cultivation column in airlift mode was proven to be successful and a high growth rate could be achieved even with a lower light intensity than the optimal. The mass production of *C. calcitrans* through a bioreactor and high growth rate have been reported (Sontaya, Worapannee, Sorawit & Prasert 2005). Many specific characteristics are thought to influence the nutritional value of micro algae, such as cell wall digestibility (Epifanio, Valenti & Turk 1981), cell size and biochemical composition (Fernandez-Reiriz, Perez-Camacho, Ferreiro, Blanco, Planas, Campos & Labarta 1989). However, no information is available on the environmental conditions for the growth of the diatom *C. calcitrans* and its effects in proximate compositions. The biochemical composition of micro algae depends on their environmental conditions, growth rates or the micro algal life cycle (Richmond 1986). The important factors used to evaluate the nutritional value of a species are growth rates, in terms of cell numbers or biomass and biochemical composition, which should be optimized in terms of vital nutrients. The

main factors controlling micro algal growth and composition are light, nutrients, temperature and pH (Tzovenis, De Pauw & Sorgeloos 1997; Zhu, Lee & Chao 1997), but other factors such as salinity can also be important for a few species (Chu, Phang & Goh 1996). The aim of the present work was to determine the effect of temperature, salinity and carbon dioxide addition on the biochemical composition and growth of the marine diatom *C. calcitrans* at different levels in a tropical commercial hatchery condition. The work mainly focuses on the total proximate composition.

Materials and methods

Micro alga culture was obtained from the algal stock collection of Central Marine Fisheries Research Institute, Kochi, India, and maintained under laboratory conditions. Experiments were performed to test the effects of temperature, salinity and carbon dioxide concentration on the growth and gross biochemical constituents of this species individually. Temperature was tested at three levels (20, 25 and 30 °C), while two salinities (25 and 35) and two CO_2 conditions [with (+) and without (−) CO_2] were used for other experiments. Carbon dioxide concentration was monitored by the free carbon dioxide method, which is based on the titration of dissolved carbon dioxide with NaOH (0.03 N), with the end-point reaction at pH 7.9 (Baumgarten, Rocha & Nienchesky 1996). Cultures were maintained using Walne medium (Walne 1974) in a Hauffkine culture flask (4 L) in a temperature-controlled algal chamber. Before each experiment, cultures remained at the determined experimental conditions for an adaptation period of approximately five generations. The cultures were started by inoculating a volume of 5–10% the total volume. Cell counts were performed daily to determine the maximum cell density and specific growth rate (K_2), which was calculated by linear regression of the $\log_2$ cell concentration on time, at the exponential growth phase (Guillard 1973). Each experiment was conducted in triplicate. Chlorophyll *a* concentration was determined at the initial and final phases of the experiment, by filtering a known culture volume on GF/F filters and extracting the pigment in 90% acetone solution for 24 h at − 20 °C. Fluorescence was then determined in the extract with a Turner Designs TD 700 fluorometer, according to Welschmeyer (1994). Light intensity was kept at 500 μmol m^{-2} s^{-1} under a photoperiod of 12 h L:12 h D. Experiments were conducted in batch cultures, which were grown until either late exponential phase or early stationary phase. Algal biomass was obtained by concentrating the three replicates from each treatment on GF/F Whatman filters, using a Millipore peristaltic pump. The samples retained on the filters were dried at 60 °C until constant weight. The filters with algae samples were stored at − 20 °C until chemical analysis. The biochemical composition of *C. calcitrans* was determined in terms of total protein, total lipids and total carbohydrates. Total lipids were extracted according to Bligh and Dyer (1959), as modified by Whyte (1987). In the lipid extract residue (polymeric fraction), total protein was determined by the Kjeldahl technique (Whyte 1987). Samples retained in GF/F filters were hydrolyzed in 10 mL 80% sulphuric acid (Myklestad & Haug 1972), and carbohydrates were determined according to Dubois, Gilles, Hamilton, Rebers and Smith (1956). Statistical analysis included one-way analysis of variance (ANOVA) and Tukey test. Total protein, total carbohydrate and total lipid percentages were transformed using arcsine before statistical analysis.

Results

The effect of temperature, salinity and carbon dioxide addition on the growth of *C. calcitrans*

Temperature had a significant effect ($P < 0.05$) on the growth rate of *C. calcitrans* (Fig. 1a) under a salinity of 25 but not under high salinity (35). Higher growth rates of *C. calcitrans* occurred when carbon dioxide was added to the cultures (Fig. 1a). The highest temperature (30 °C) caused the growth rate to be lower at salinity 25, with no addition of carbon dioxide. Temperature showed no effect ($P > 0.05$) on the maximum cell concentration (Fig. 1b), although a visible trend of high values at 25 °C was observed when carbon dioxide was added. Carbon showed lower values at 30 °C (Fig. 1c), while chlorophyll per cell (Fig. 1d) was not affected by temperature or any other factors tested. Salinity (25–35) had no significant effect ($P > 0.05$) on *C. calcitrans* growth, maximum cell density, biomass and chlorophyll per cell (Fig. 1a–d). However, an affinity of higher growth and biomass as well as low cell concentration was observed at the lower salinity.

The effect of temperature, salinity and carbon dioxide addition on the biochemical composition of *C. calcitrans*

Temperature seems to influence the biochemical composition. At temperatures 20 and 25 °C, lipids

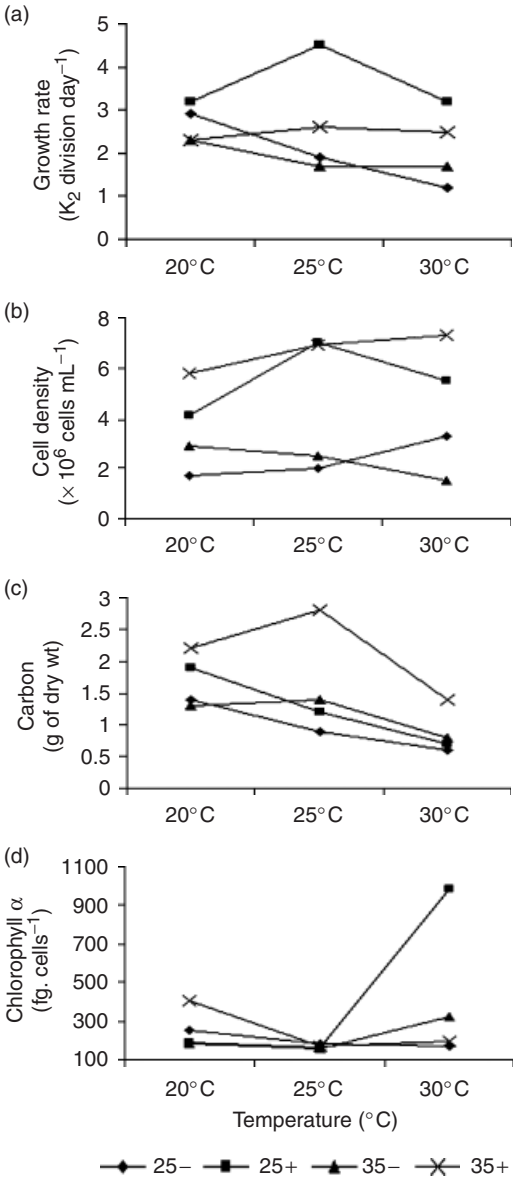

Figure 1 (a) Growth rate, (b) cell density, (c) carbon and (d) chlorophyll *a* under different temperatures, salinities (25 and 35) and with (+) and without (–) addition of CO_2.

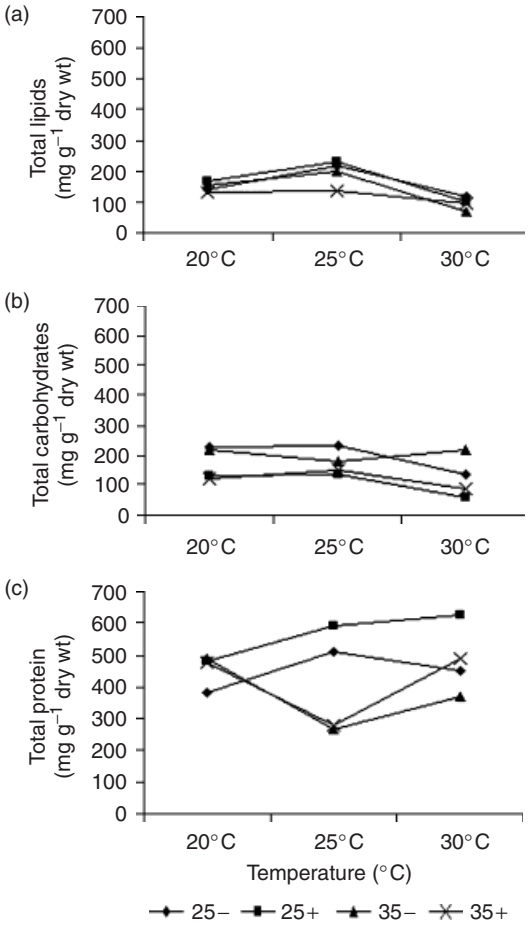

Figure 2 (a) Total lipids, (b) carbohydrates and (c) protein under different temperatures, salinities (25 and 35) and with (+) and without (–) addition of CO_2.

and carbohydrates were higher than at 30 °C under salinity 25 (Fig. 2a and b). Protein was not significantly affected by temperature, but an affinity for lower values was observed at 25 °C under salinity 35 (Fig. 2c). The effect of carbon dioxide on the biochemical composition of *C. calcitrans* is shown in Fig. 2a–c. An increase in protein content (Fig. 2c) and a decrease in carbohydrates (Fig. 2b) were noted.

Discussion

The effect of temperature on the growth rate of micro algae has been observed in other species. Significant increases in growth rates of *Chaetoceros pseudocurvisetus*, *Skeletonema hantzschii*, *Skeletonema costatum* with a maximum at 25 °C were observed by Yoshihiro and Takahashi (1995). Renaud, Thinh, Lambridis and Parry (2002) attributed the higher growth rate of *Chaetoceros* sp. to an increase in temperature from 25 to 30 °C. Fogg and Thake (1987) stated that a lower micro algae growth rate could be a result of the increase in respiration due to rise in temperature above the species' optimum level. It is possible that all these effects are related to the results observed in growth rate, and therefore in cell density and biomass in this work. As the results suggest, the adequate

temperature for *C. calcitrans* is between 20 and 25 °C, under the conditions used in these experiments.

Salinity (25–35) had no significant effect on growth, maximum cell density, biomass and chlorophyll per cell, although a tendency of higher growth, biomass and lower cell density was observed at the lower salinity. The observed contrast between higher growth rate and lower maximum cell density could be explained by a limitation in some nutritional factor not determined at the lowest salinity (25), as the medium of this salinity was obtained by dilution of the seawater. Further tests are needed to determine the cause of such results.

Higher growth rates occurred when carbon dioxide was added to the cultures, indicating that although other factors may be sufficient, the nutrient can limit the algal growth. Increases in growth rate have been observed in other micro algae species with carbon dioxide addition (Olaizola, Duerr & Freeman 1991). It was also reported that addition of carbon dioxide to algal culture extends the exponential phase, which is important in the hatchery system, as it provides maximum nutritional value for aquatic animals (Fabregas, Otero, Dominguez & Patino 2001).

In the present study, lipids and carbohydrates were high at low temperatures, whereas the protein value was low at 25 °C. According to Renaud, Zhou, Parry, Thinh and Woo (1995), the maximum lipid content coincides with the optimal range in growth temperature in many species and varies at temperatures below and above this range. Another investigation showed a higher lipid content at 25 °C for *Chaetoceros* sp., while *Rhodomonas* sp., *Cryptomonas* sp. and *Isochrysis* sp. showed higher concentrations between 27 and 30 °C (Renaud *et al.* 2002). All the species studied showed a significantly lower protein content at temperatures above 27 °C. Carbohydrates in *Chaetoceros* sp. were significantly higher between 25 and 30 °C and became lower at higher temperatures. In general, the results of biochemical composition of *C. calcitrans* are in accordance with other similar works. Lipids and carbohydrates are considered as stored energy products (Thompson, Guo & Harrison 1992) and their decrease can negatively affect the growth and metabolic activities of cells.

The present study suggests that temperatures between 20 and 25 °C could be used to optimize the nutritional value of *C. calcitrans* due to the higher lipid and carbohydrate and adequate protein content under these conditions. Higher levels of carbohydrates are reported to produce higher growth of *Ostrea edulis* juveniles (Enright, Newkirk, Craigie & Castell 1986) and larvae of *Patinopecten yessoensis* (Whyte *et al.* 1989).

The effects of salinity on proximate composition of algae are shown in Fig. 2a–c. Protein content was low at a salinity of 35, while lipids and carbohydrate increased slightly by mineral fraction. Although many species of micro algae are tolerant to great variations in salinity, their chemical composition can be affected (Brown, Jeffrey & Garland 1989; Roessler 1990). Protein, lipids and carbohydrates seem slightly affected by a wide range of salinity for most micro algae species (Richmond 1986). However, in some species, increases in ash and lipid content were observed at higher salinity (Ben-Amotz, Fishler & Schneller 1987). Fabregas, Herrero, Abalde and Cabezas (1985) reported a decrease in the protein content with an increase in salinity. The result shows that a salinity of 25 is optimum for *C. calcitrans* in terms of growth and chemical composition.

The effect of carbon dioxide on the biochemical composition of *C. calcitrans* is shown in Fig. 2a–c. A decrease in carbohydrate (Fig. 2b) and an increase in protein content (Fig. 2c) were noted when carbon dioxide was added to the culture. Brown, Jeffrey, Volkman and Dunstan (1997) noticed an increase (100%) in protein content when cultures were enriched with 1% carbon dioxide in many species in different groups of micro algae. Lipids and carbohydrates, on the other hand, were not affected. In *Phaeodactylum tricornutum*, the protein content was increased with carbon dioxide addition (Chrismadha & Borowitzka, 1994). Chu *et al.* (1996) observed increases in lipids and carbohydrates at protein expenses in the diatom *Nitzschia inconspicua*, when the culture was enriched with 5% (v/v) of carbon dioxide. In the present work, *C. calcitrans* apparently directed the extra-assimilated carbon mainly to protein synthesis, indicating a positive effect on cell physiology. Probably, the cells were investing the excess of carbon assimilated much more in protein synthesis and growth rather than lipids and carbohydrates as reserve substances in micro algae.

According to the results, a salinity of 25, temperature between 20 and 25 °C and addition of carbon dioxide seems more adequate for enhanced growth of *C. calcitrans* and high biochemical composition in terms of protein, lipids and carbohydrates. The system could be useful for the high algal production and successful operation of a hatchery system.

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