Article ID: 1003—6482（2000）04—0053—10

EFFECTS OF SALINITY ON THE GROWTH AND FATTY ACID COMPOSITION OF SIX STRAINS OF MARINE DIATOMS[1]

LIANG Ying MAI Kangsen SUN Shichun
HUANG Guoxiao HU Haiyan
(*Aquaculture Research Lab, Ocean University of Qingdao, Qingdao* 266003)

Abstract

The effect of salinity (18‰, 28‰ and 38‰) on the growth and fatty acid composition of six strains of marine diatoms *Chaetoceros gracilis* (B13), *Cylindrotheca fusiformis* (B211), *Phaeodactylum tricornutum* (B114, B118 and B221) *and Nitzschia closterium* (B222) was investigated. The relative growth rate showed an increase in B13 and a decrease in B114 and B222 with the increase of salinity. The dry weight of B13 increased with the increase of salinity and reached its highest value (0.21) at the highest salinity (38‰). The dry weight of B114, B118, B221 and B222 both reached their highest values (0.26, 0.32, 0.40 and 0.30, respectively) at salinity 28‰. No significant difference was observed in B211 (0.35~0.36). The major fatty acids of the 6 strains were 16:0, 16:1(n-7) and 20:5(n-3). B211 also had a high percentage of 20:4n-6 (5.6~7.4%). B13 also had a high percentage of 14:0 (20.0~ 30.9%). Saturated fatty acids decreased with the increase of salinity in B211, B221 and B222, and reached their highest values in 18‰ in B211 (41.7%), B221 (37.6%) and B222 (31.7%), and in 28‰ in B13 (48.1%), B114 (33.0%) and B118 (31.0%). Monounsaturated fatty acids increased in B114, B118, B221 and B222 while decreased in B13 and B211 with the increase of salinity. Polyunsaturated fatty acids had their highest values in 18‰ in B13 (31.3%), B114 (19.6%), B118 (23.4%) and B222 (18.6%), and in 28‰ in B211 (25.1%) and B221 (16.3%).

Keywords: Diatom, salinity, relative growth rate, dry weight, fatty acid

CLC number: Q949.27 Q547 P731.12 **Document code:** A

INTRODUCTION

Fatty acids play an important role in animal nutrition as energy sources, membrane constituents, and metabolic intermediates. Many marine animals cannot synthesize the long-chain

[1] Project supported by the Hi-Tech "863" programs of China Ministry of Science and Technology (863-819-02-01)

polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); they must be obtained from food. Insufficiency of EPA and DHA in the diet of marine animals can result in serious problems such as malnutrition, abnormality development, stunted growth and even death in aquaculture (Yongminitchai and ward, 1989; Renaud et al., 1991; Reitan et al., 1994). One way to solve this malnutrition problem is to provide them with polyunsaturated fatty acids (PUFAs), especially EPA and DHA. Rotifers, copepods and brine shrimps can be reared on microalgae rich in EPA and DHA. Although EPA and DHA are not essential to some animals, addition of these two fatty acids to their food can increase growth rate and survival rate (Yongminitchai and ward, 1989; Renaud et al., 1991; Reitan et al., 1994).

The biochemical composition of microalgae changes when culture conditions are changed. The relative EPA content of a certain algal strain is not only determined by its heredity, but also can be modified under different growing conditions (Teshima et al., 1983; Thompson et al., 1990; Renaud et al., 1991; Yongmanitchai & Ward, 1991; Reitan et al., 1994; Zhou et al., 1996). Salinity often has a dramatic effect on the biochemical profile of most microalgae (Cohen & Heimer, 1988; Al-Hasan et al., 1990; Yongmanitchai & Ward, 1991; Floreto et al, 1993). In this paper we present data on the growth and fatty acid composition of six strains of marine diatoms (obtained from the Microalgae Culture Center (MACC), Ocean University of Qingdao) cultured at different salinities, in order to establish for each strain the most appropriate salinity from the point of view of its nutritional for aquaculture.

MATERIALS AND METHODS

1. Microalgal strains

The following 6 strains of marine diatoms were obtained from the Microalgae Culture Center (MACC), Ocean University of Qingdao.

Chaetoceros gracilis MACC/B13
Cylindrotheca fusiformis MACC/B211
Phaeodactylum tricornutum MACC /B114
Phaeodactylum tricornutum MACC /B118
Phaeodactylum tricornutum MACC /B221
Nitzschia closterium MACC /B222

2. Culture conditions

Cultures were carried out using 3-L flasks in f/2 medium (Jing, 1995) at 22±1℃; Successive aeration and 5000lux light intensity were supplied (using fluorescent tubes as light source). The salinity of culture media was adjusted to 18‰, 28‰, and 38‰, respectively. The relative growth rate K was calculated during logarithmic phase as:

$$K = (\log_{10} N_1 - \log_{10} N_0) / T_1 - T_0$$

where N_1 = cell density at time 1 (T_1) and N_0 = cell density at time 0 (T_0)

3. Harvest

The cell density for each microalgal culture was determined every day with a haemocytometer. The cultures were centrifuged (4000 rev/m) at the late exponential phase. Vacuum dried samples were preserved at -40℃ in test tubes filled with nitrogen until analysis.

4. Fatty acid analysis

Fatty acids were analyzed on a HP5890II gas chromatograph fitted with a carbowax capillary column (30m × Φ0.25mm) by the modified method of Volkman et al. (1989). High purity N_2 was the carrier gas at a flow rate of 2ml min^{-1}, injector and detector temperature 280℃. The oven was programmed from 150℃ to 200℃ at 15℃ min^{-1}, then to 250℃ at 2℃min^{-1} and held until all peaks had eluted. Fatty acid methyl esters were identified by comparing the retention times of the experimental samples to those of known standards.

RESULTS

1. The relative growth rate and harvested biomass (expressed in dry weight)

The effect of salinity on the relative growth rate and dry weight of the six diatom strains were shown in Table 1.

Table 1　The harvested biomass and dry weight of the six diatom strains

Microalgal strains	Salinity	Relative growth rate	DW(g/l)
B13	18	0.27±0.00	0.06±0.02
	28	0.27±0.04	0.08±0.02
	38	0 29±0.01	0.21±0.09
B114	18	0.36±0.05	0.25±0.04
	28	0.35±0.01	0.26±0.01
	38	0.22±0.01	0.20±0.01
B118	18	0.32±0.02	0.27±0.05
	28	0.37±0.02	0.32±0.11
	38	0.35±0.01	0.29±0.02
B221	18	0.27±0.02	0.24±0.02
	28	0.31±0.01	0.40±0.09
	38	0.27±0.02	0.16±0.02
B222	18	0.35±0.02	0.29±0.07
	28	0.34±0.04	0.30±0.02
	38	0.29±0.00	0.25±0.05
B211*	18	—	0.35±0.04
	28	—	0.35±0.01
	38	—	0.36±0.04

* — not detectable

The relative growth rate showed an increase in B13 and a decrease in B114 and B222 with the increase of salinity, and reached their highest values at salinity 38‰ in B13 (0.29), at salinity 18‰ in B114 (0.36) and B222 (0.35), at salinity 28‰ in B118 (0.37) and B221 (0.31).

The dry weight of B13 increased with the increase of salinity and reached its highest value (0.21) at the highest salinity (38‰). The dry weight of B114, B118, B221 and B222 all reached their highest values (0.26, 0.32, 0.40 and 0.30, respectively) at salinity 28‰. No significant difference was observed in B211 (0.35~0.36).

2. Fatty acid composition

The fatty acid composition of the six strains of marine diatoms cultured at different salinity was given in Table 2 and Figure 1~3.

Table 2 Fatty acid composition of six diatom strains cultured at different salinities (expressed in percentage of total fatty acid)

Fatty acid	**Chaetoceros gracilis** MACC B13			*Cylindrotheca* sp. MACC B211		
	18	28	38	18	28	38
Saturates						
14:0	20.0±0.5	30.9±2.1	20.0±0.4	6.8±2.2	4.9±0.6	4.2±0.1
15:0	0.8±0.1	0.4±0.3	0.6±0.1	0.6±0.1	0.5±0.1	0.6±0.2
16:0	12.8±2.1	16.4±0.4	10.6±0.2	34.1±0.1	32.1±0.4	32 3±2.6
18:0	0.8±0.1	0.4±0.3	0.6±0.1	0.2±0.2	0.5±0.1	0.6±0.1
Sum%	34.4	48.1	31.8	41.7	38.0	37.7
Monounsaturates						
16:1(n-9)	1.2±0.1	0.8±0.8	4.6±0.1	1.5±0.5	1.0±0.5	0.6±0.1
16:1(n-7)	28 5±1.6	28.3±0 4	23.1±0.4	27.2±1.2	25.4±3.6	24.8±0.1
18.1(n-9)	1.2±0.1	1.0±0.0	1.2±0.1	2.0±0.4	3.6±0.5	3.8±0.5
18:1(n-7)	1.5±0.3	1.4±0.0	1.2±0.1	0 5±0.1	0.6±0.1	0.6±0.1
Sum%	32.4	31.5	30.1	31.2	30.6	29.8
Polyunsaturates						
16·2 (n-7)	0.9±0.1	—	0.7±0.1	1.0±0.0	0.6±0.1	0.7±0.4
16:2 (n-4)	1.8±0 1	1.9±0.6	1.5±0.1	1.0±0.0	0.7±0.0	0.6±0.1
16:3 (n-4)	2.1±0.2	1.5±0.6	1.3±0.1	2.9±0.1	1.5±1.0	1.9±0.1
16:4 (n-1)	0.1±0.0	—	0.2±0.1	0.6±0.2	0.4±0.1	0.5±0.0
18 2 (n-6)	1.5±0.2	1.2±0.1	1.9±0.1	1.7±0.4	2.3±0.1	2.5±0.1
18:3 (n-6)	9.3±4.5	4.9±0.9	3.3±0.2	3.4±1.6	3.6±0.4	2.3±0.6
18:3 (n-3)	0.2±0.1	1.2±0.0	0.1±0.0	0.7±0.1	0.8±0.1	0.8±0.1
18:4 (n-3)	0.5±0.1	0.2±0.1	0.5±0.1	0.1±0.1	0.3±0.1	0.3±0.0
20 4 (n-6)	4.5±0.6	2.8±0.5	7.0±0.2	5.6±0.6	7.4±1.3	7.4±0.1
20:4 (n-3)	0.1±0.0	—	0.1±0.1	—	0.2±0.1	0.2±0.1
20:5 (n-3)	8.7±1.3	4.5±0.4	6.2±0.1	4.9±1.2	6.7±0.8	6.8±0.8
22:5 (n-3)	0.3±0.3	0.1±0.1	0.3±0.1	—	0.2±0.0	0.2±0.1
22.6 (n-3)	1.3±0.2	0.4±0.0	2.4±0.1	0.5±0.2	0.4±0.0	0.4±0.1
Sum%	31.3	18.7	25.5	22.4	25.1	24.6

Fatty acid	*Phaeodactylum tricornutum* MACC B118			*Phaeodactylum tricornutum* MACC B114		
	18	28	38	18	28	38
Saturates						
14:0	3.7±0 7	3.7±0.6	4.8±1 1	3.6±0.0	3.8±0.7	2.5±0.2
15:0	0.4±0.2	0.6±0.1	0.5±0 1	0.2±0.1	0.6±0.2	0 4±0.1
16 0	21.7±1.0	25.9±1.0	23.0±0.4	25.9±1.4	27.9±0.4	23.6±0.6
18.0	1.2±0.0	0.8±0.1	1.1±0.9	0.9±0.1	0.7±0.1	0.6±0.1
Sum%	27.0	31.0	29.4	30.6	33.0	27.1
Monounsaturates						
16 1(n-9)	0.2±0.0	0.8±0.1	1.2±0.4	0.9±0.0	1.1±0.1	4.4±0.1
16.1(n-7)	33.2±1.0	39.4±1.3	40.0±6.4	34.1±2.3	42.5±0.1	40.8±0.2
18.1(n-9)	5.1±0 3	2.2±0.1	4.5±0.9	3.3±1 1	2.8±0.1	2.5±0.0
18.1(n-7)	1.4±0.2	1.3±0.0	1.1±0 1	1.2±0.1	1.3±0.1	2.0±0.3
Sum%	39.9	43.7	46.8	39.5	47.7	49.7
Polyunsaturates						
16:2 (n-7)	0.8±0.0	0.8±0.4	0.8±0 1	0.4±0.1	0.4±0.0	0.5±0.1
16.2 (n-4)	1.3±0.2	0.9±0.1	0.9±0.1	0 6±0.4	0.7±0.2	0.7±0.0
16.3 (n-4)	2.0±0 3	1.4±0.1	1.7±0.5	1.0±0.5	1.1±0.1	1.3±0.3
16:4 (n-1)	0.9±0.2	0.7±0.3	0.8±0.1	0.5±0.1	0.5±0.1	0.5±0.0
18.2 (n-6)	1.6±0.1	1.1±0.1	1.0±0.0	1.2±0.5	1.3±0.4	1.1±0.1
18:3 (n-6)	3.3±0 8	4.0±1.8	3.7±0.6	3.5±3.1	2.1±1.3	1.5±0 3
18:3 (n-3)	0.4±0.0	0.5±0.1	0.5±0.1	1.0±0.1	0.1±0.0	1.0±0.1
18:4 (n-3)	0.8±0.1	0.4±0.2	0.3±0.0	0.5±0.1	0.4±0.1	0.2±0.0
20:4 (n-6)	1.5±0.3	1.2±0.4	1.1±0.3	0.9±0.6	0.7±0.1	1 0±0.1
20:4 (n-3)	0.5±0.0	0.5±0.0	0.5±0 4	0.5±0.0	0.3±0.1	0.4±0.1
20:5 (n-3)	8.9±1 3	8.9±0.1	7 2±2.1	6.9±2.2	5.4±1.9	7.3±1.5
22 5 (n-3)	0.6±0.3	1.2±0.1	1.0±0.6	1.6±0.3	0.9±0.4	1.3±0.4
22 6 (n-3)	0.8±0.3	0.8±0.2	0 4±0.3	1.0±0.5	0.6±0.2	0.6±0.1
Sum%	23.4	22.4	19.9	19.6	14.5	17.4

Fatty acid	*Phaeodactylum tricornutum* MACC B221			*Nitzschia closterium* MACC B222		
	18	28	38	18	28	38
Saturates						
14:0	5.3±0.5	3.8±0.6	3.4±0.4	4.5±0.5	3.4±0.1	2.6±0.4
15:0	0.3±0.1	0.3±0.1	0 4±0.1	0.4±0.1	0.5±0.2	0.4±0.0
16:0	31.1±1.2	27.4±1.8	25.5±2.1	25.8±2.8	24.1±1.1	24.8±0.4
18:0	0.9±0.1	0.7±0.0	0.7±0.1	1.0±0.3	0.6±0.1	0 7±0.2
Sum%	37.6	32.2	30.0	31.7	28.6	28.5
Monounsaturates						
16:1(n-9)	0.6±0.1	3.5±3.3	0.2±0.0	0.8±0.4	3.8±4.1	2.0±1.6
16:1(n-7)	40.6±2.3	40.0±4.0	44.5±2.0	39.1±1.3	37.7±0.7	45.8±3 5
18 1(n-9)	3.3±0.2	3.9±0.4	3.8±0.6	4.8±1.6	4.9±0.4	4.0±0.9
18:1(n-7)	1.3±0.1	1.3±0.1	2.5±0.3	1 4±0.2	1.5±0.0	2.9±0.6
Sum%	45.8	48.7	51.0	46.1	47.9	54.7
Polyunsaturates						
16:2 (n-7)	0.5±0.0	0.5±0.1	0 5±0.1	0.7±0.2	0.5±0.4	1.1±0.0
16:2 (n-4)	0.7±0.1	0.7±0.2	0.6±0.2	0.7±0.2	0.7±0.2	0.6±0.0
16:3 (n-4)	1.1±0.1	1.0±0 1	1.1±0.1	1.4±0.6	1.2±0.1	1.1±0.0
16:4 (n-1)	0.3±0.0	0.3±0.0	0.5±0.0	0.7±0.3	0.8±0.1	0.6±0.1
18:2 (n-6)	0.9±0.2	1.1±0.2	0.9±0.1	0.6±0.3	0.8±0.2	0.6±0.1
18:3 (n-6)	1.1±0 2	2.3±0.3	0.8±0.2	1.6±0.1	2.3±0.2	2.1±0.4
18:3 (n-3)	1.0±0.1	0 8±0.0	0.8±0.0	0.3±0.1	0 3±0.0	0.3±0.0
18.4 (n-3)	0.4±0.0	0.3±0.0	0.3±0.1	0.3±0.0	0.3±0.1	0.2±0.0
20:4 (n-6)	0.7±0.2	0 8±0.1	1.0±0.2	0.5±0.2	0.4±0.0	0.3±0.0
20:4 (n-3)	0.4±0.1	0.5±0.1	0.3±0.1	0.4±0.1	0.2±0.0	0.2±0.1
20:5 (n-3)	6.0±0.6	6.6±0.7	6.6±0.9	9.0±2.8	8.1±1 1	5.9±2.1
22:5 (n-3)	0.7±0.1	1 0±0.0	1.0±0.1	1.8±0.6	1.5±0.3	1.0±0.6
22:6 (n-3)	0.4±0.0	0.4±0.0	0.3±0.0	0.6±0.1	0 4±0.0	0.3±0.1
Sum%	14.2	16.3	14.7	18.6	17.5	14.3

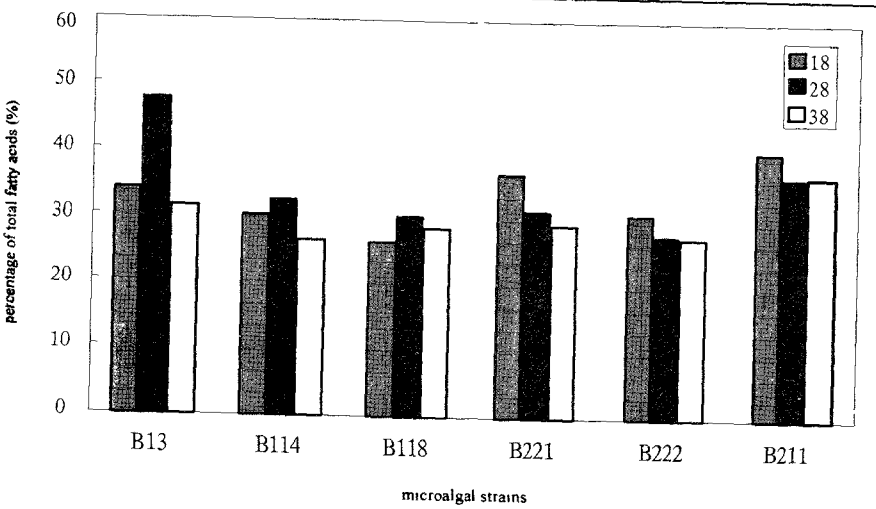

Fig 1 Effects of different salinity on the total saturated fatty acids of six diatom strains

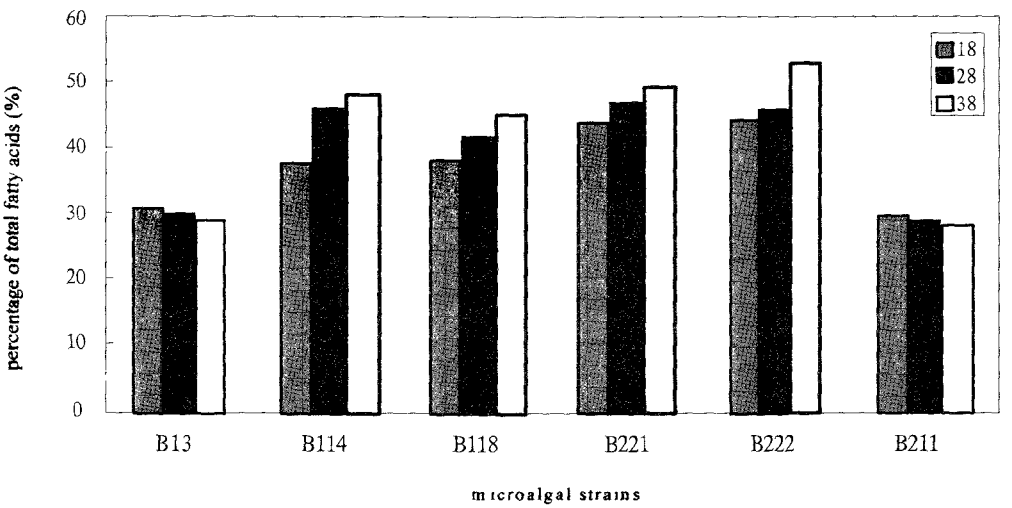

Fig 2 Effects of different salinity on the total monounsaturated fatty acids of six diatom strains

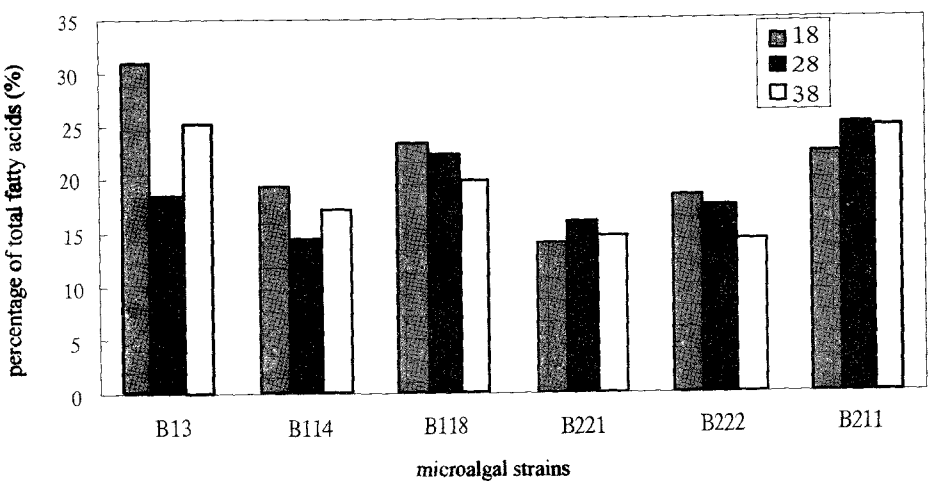

Fig. 3 Effects of different salinity on the total polyunsaturated fatty acid of six diatom strains

The major fatty acids present in B13 were 14:0 (20.0~ 30.9%), 16:0 (10.6~16.4%), 16:1n-7 (23.1~28.5%), 18:3n-6 (3.3~9.3%), 20:4 n-6 (2.8~7.0%) and 20:5n-3 (4.5~8.7%). The total saturated fatty acids reached its highest value (48.1%) at salinity 28‰. The total monounsaturated fatty acids and polyunsaturated fatty acids both reached their highest values (32.4% and 31.3%, respectively) at the lowest salinity (18‰).

In the case of B211, the major fatty acids were 14:0 (4.2~6.8%), 16:0 (32.1~ 34.1%), 16:1n-7 (24.8~ 27.2%), 20:4n-6 (5.6~7.4%) and 20:5n-3 (4.9~6.8%). The increase of salinity caused a decrease in total saturated fatty and total monounsaturated fatty acids; they both reached their maximal values at salinity 18‰ (41.7% and 21.2%, respectively). The total polyunsaturated fatty acids increased when salinity increased from 18‰ to 28‰ followed by a slight decrease at salinity 38‰.

As regard to B114 and B118, the major fatty acids were 16:0, 16:1n-7 and 20:5n-3. But the levels of each of them changed with the medium salinity of the culture. The total monounsaturated fatty acids increased while the total polyunsaturated fatty acids had a decrease tendency with the increase of salinity. The total saturated fatty acids reached their highest values (31.0% and 33.0%, respectively) at salinity 28‰.

The major fatty acids in B221 and B222 were 16:0, 16:1n-7 and 20:5n-3.The two strains show an increase in the levels of total monounsaturated fatty acids and a decrease in the levels of total saturated fatty acids with the increase of salinity. Polyunsaturated fatty acids did not show any significant difference in different salinities.

DISCUSSION

A wide range of relative fatty acid content has been reported for different diatom species (Orcutt & Patterson, 1975; Volkman et al., 1989; Thompson et al., 1990; Yongmanitchai & Ward, 1991; Viso & Marty, 1993; Dunstan et al., 1994; Reitan et al., 1994; Zhukova & Aizdaicher, 1995; Grima et al., 1996; Zhou et al., 1996). This is connected with the wide use of diatoms in aquaculture and their worldwide distribution. The results show that almost all diatoms contained high proportions of 14:0, 16:0, 16:1(n-7) and 20:5 (n-3) fatty acids. C_{18} and C_{22} PUFAs were minor constituents. Our experiment also reached this conclusion. We also found that the proportion of 14:0 in B13 is higher than that of the other 5 diatom strains, this could be due to different genus and different species.

The results presented in this work show the differences in the growth and fatty acid composition among strains as well as among different medium salinity. Salinity often has a significant effect on fatty acid composition of most microalgae (Cohen & Heimer, 1988; Al-Hasan et al 1990; Yongmanitchai & Ward, 1991; Floreto et al, 1993). For example, the EPA content in *Porphyridium cruentum* decreased from 37.5% to 18.9% when sodium chloride concentrations increased from 0.25g/l to 2.0g/l. The results of Yongmanitchai and Ward (1991) showed that EPA content did not show any difference when sodium chloride concentrations were 0~5g/l, but EPA

content decreased significantly when sodium chloride concentrations are higher than 5g/l. But not all microalgae has this trend; the results studied by Teshima (1983) showed that the alteration of salinity within the range of 4-30‰ did not result in the variation in the EPA and other fatty acid levels of *Chlorella* lipids. In our experiment, salinity has an evident effect on fatty acid composition of the six strains of marine diatoms. Saturated fatty acids decreased with the increase of salinity in B211, B221 and B22; Monounsaturated fatty acids increased in B114, B118, B221 and B222 while decreased in B13 and B211 with the increase of salinity; Polyunsaturated fatty acids had their highest values in 18‰ in B13, B114, B118 and B222, and in 28‰ in B211 and B221. It is not easy to compare data on the biochemical composition of microalgae. Differences in the culture conditions, in the analytical methods or in the growth phase harvested make it difficult to compare the results presented by different authors.

In conclusion, the biochemical properties of microalgae change when the culture conditions (water temperature, salinity, light intensity, medium composition etc.) change (Teshima et al., 1983; Thompson et al., 1990; Renaud et al., 1991; Yongmanitchai and Ward, 1991; Reitan et al., 1994; Zhou et al., 1996). This allows us to select the culture conditions to produce microalgal species with a balanced fatty acid composition for the larvae and spat of bivalve mollusks.

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盐度对六株硅藻生长及脂肪酸组成的影响

梁　英　麦康森　孙世春　黄国效　胡海燕

(青岛海洋大学教育部水产养殖重点实验室 青岛 266003)

摘　要

在温度为 22±1℃，盐度为 18‰、28‰和 38‰的条件下，用 F/2 培养基对青岛海洋大学微藻种质库保存的 6 株硅藻（纤细角毛藻 *Chaetoceros gracilis* B13；筒柱藻 *Cylindrotheca fusiformis* B211; 三角褐指藻 *Phaeodactylum tricornutum* B114, B118, B221; 新月菱形藻 *Nitzschia closterium* B222）进行培养，在指数生长期末期进行收获，测定了 6 株硅藻的生长及脂肪酸组成。实验结果表明：盐度对六株硅藻的生长及脂肪酸组成均有影响，但作用结果因种而异。B13 的相对生长率随着盐度的增加而增加；B114 和 B222 的相对生长率随着盐度的增加而降低。B13 的干重

随着盐度的增加而增加，在盐度为 38‰时达到最大值（0.21）；B118、B114、B221 和 B222 的干重均在盐度为 28‰时达到最大值；盐度对 B211 的干重影响不明显（0.35~0.36）。六株硅藻的主要脂肪酸为 16：0、16：1（n−7）和 20：5（n−3），B211 还含有较多的 20:4n−6 (5.6~7.4%)，B13 含有较多的 14:0 (20.0~ 30.9%)。B211、B221 和 B222 的饱和脂肪酸总合随着盐度的增加而降低，在盐度为 18‰时达到最大值（占总脂肪酸的百分比分别为 41.7%、37.6%和 31.7%）。而 B13、B118 和 B114 的饱和脂肪酸总合在盐度为 28‰时含量最高（分别为 48.1%、31.0%和 33.0%）。B118、B114、B221 和 B222 的单不饱和脂肪酸总合随着盐度的增加而增加，而 B13 和 B211 的单不饱和脂肪酸总合随着盐度的增加而降低。B13、B118、B114 和 B222 的多不饱和脂肪酸总合在 18‰时含量最高（分别为 31.3%、23.4%、19.6%和 18.6%）。B211 和 B221 的多不饱和脂肪酸总合在 28‰时含量最高（分别为 25.1%和 16.3%）。

关键词： 硅藻，盐度，相对生长率，干重，脂肪酸