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Effects of Environmental Characteristics of Aquaculture Sites on the Quality of Cultivated Newfoundland Blue Mussels (*Mytilus edulis*)

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This study examined (1) the effects of seasonal and annual environmental characteristics (temperature, chlorophyll content, salinity, microbial water quality, and algal lipid composition) in four aquaculture locations on the quality (meat content, shell size, and microbial and lipid compositions) of cultivated blue mussels and (2) the optimum harvest time for premium-quality mussels. Seasonal and annual temperature fluctuation of the seawater in the aquaculture sites significantly ($P < 0.05$) affected their salinity, microbial content, and algal fatty acid compositions, which in turn affected the quality attributes of cultivated mussels. The optimum growth period in terms of meat content (4−5 g) and shell size (50−60 mm) can be achieved within 1 year of cultivation. Because the cultivated mussels examined in this study never reached the maximum microbial load limits (10^5 or log 5 colony-forming units/g of meat), they can be harvested throughout the year. Meanwhile, no significant ($P > 0.05$) annual changes were observed in total omega-3 polyunsaturated fatty acids content of cultivated mussels. Nonetheless, the optimum harvest time may be April−June for eicosapentaenoic acid (EPA) rich mussels and September−October for docosahexaenoic acid (DHA) rich mussels in terms of fatty acid proportions; however, June may be the best month for the highest concentrations of both EPA and DHA.

KEYWORDS: Chemical and physical hydrography; blue mussels (*Mytilus edulis*); aquaculture sites; fatty acid composition; quality; meat content; shell size; optimum harvest; DHA; EPA

INTRODUCTION

Blue mussels (*Mytilus edulis*) are cultivated and produced mainly by China, Spain, France, Britain, Ireland, Greece, Australia, Chile, and New Zealand. World production of cultured mussels in 2000 was 1.3 million metric tonnes, of which 80% were blue mussels. Canadian production of blue mussels in 2000 was only 21287 tonnes. Although Canadian and therefore Newfoundland cultured blue mussel input to the international market is small, blue mussels are the single largest cultured shellfish product of Newfoundland, with strong impacts on its economy (*1*, *2*). Newfoundland's cold waters and the geographic nature of the province with thousands of sheltered marine sites provides promising aquaculture areas for farming blue mussels. Experimental farming trials started in the 1970s, and full production began in the 1990s. More than 92% of blue mussels in Newfoundland are cultivated in the Notre Dame Bay area (*1*, *3*).

Monitoring environmental characteristics of aquaculture sites (temperature, chlorophyll content, salinity, microbial water quality, and algal fatty acid composition) and their effects on the quality of cultivated bivalves (meat content, shell size, and microbial and lipid compositions) in various seasons and years will provide the aquaculture industry with essential information for proper site selection and optimum harvest time (*4*−*6*). Slabyj et al. (*7*) reported seasonal effects on proximate composition and quality of wild and cultured blue mussel meats. Krzynowek and Wiggin (*8*) found that seasonal variations in the proximate composition of cultured blue mussels affect the quality of cooked products during storage at −20 °C. The authors suggested that mussels in the northeastern United States should be harvested in late spring or early summer for optimum quality. Krzynowek (*9*) reported seasonal variations in cholesterol levels in blue mussels. Dolmer (*10*) observed that season, salinity, and current speed affected the growth of cultured blue mussels. More recently, Orban et al. (*11*) evaluated seasonal changes in chemical composition in Italian cultured blue mussels and found that the fatty acid composition of mussels changes depending on harvest season.

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Various nutritional and clinical studies have indicated that omega-3 polyunsaturated fatty acids (n-3 PUFA) can reduce the risk of many clinical disorders, including cardiovascular diseases (*12*, *13*). In addition, some specific fatty acids can be identified in a given microorganism and thus may serve as lipid biomarkers. Iso- and anteiso-branched-chain fatty acids have been detected in the well-known foodborne pathogen *Listeria monocytogenes* (*14*). Cyclopropanoic acid has been observed in Gram-positive and Gram-negative bacteria, but not in fungi or animal biomarkers. Furthermore, hydroxy fatty acids (3-OH), such as 3-hydroxymyristic acid, have been detected in Gram-negative bacteria such as *Haemophylus* (*15*). Therefore, the fatty acid composition of the aquaculture site and cultivated mussels can provide useful information about their microbial populations and the quality of mussels.

The selection of Newfoundland blue mussel cultivation sites and harvest times has generally been based on practical experience, market demands, and fragmented research studies. It appears that there is no comprehensive up-to-date study in the literature examining the effects of environmental characteristics on the quality of cultivated Newfoundland blue mussels to aid in the selection of proper aquaculture sites or optimum harvest times. Premium quality cultivated mussels should reach market shell size (5−6 cm) in the shortest possible period of time, with the highest amount of meat and nutritional fatty acids, docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), and the lowest total bacterial counts. Therefore, the objectives of this study were to (1) measure seasonal and annual variations in salinity, chlorophyll *a* content, temperature, microbial content of the seawater, and plankton fatty acid composition at two blue mussel aquaculture sites (two stations per site) and (2) examine variations in meat content, shell size, microbial content, and lipid composition during the growth of blue mussels at the four stations.

MATERIALS AND METHODS

Materials. Compressed air, hydrogen, and UHP helium were obtained from Canadian Liquid Air Ltd. (St. John's, NL). Marine agar was purchased from Becton Dickinson Microbiology systems (Sparks, MD). PUFA 1, PUFA 3, Supleco 37 component FAME mixture, and bacterial acid methyl ester mixture were obtained from Supelco, Canada Ltd. (Oakville, ON). GLC-461 standard fatty acid methyl ester preparation was purchased from Nu-Check-Prep, Elysian, MN. All other chemicals were of ACS grade or better quality.

Cultivation of Mussels and Sampling Intervals. Blue mussels used in this study were cultivated in June 2000 at two aquaculture sites with two stations per site, Fortune Harbor and Charles Arm. The two sites are located in the Notre Dame Bay area north of St. John's, NL, Canada. The geographic locations of the stations in each site were 49° 21.617′ N, 055° 17.0106′ W (Charles Arm station I); 49° 20.849′ N, 055° 16.722′ W (Charles Arm station II); 49° 30.942′ N, 055° 15.186 W (Fortune Harbor station I); and 49° 31.693′ N, 055° 16.545 W (Fortune Harbor station II). The mussels were cultured on long ropes anchored at both sides and supported by floating buoys. Mussel spat (12-month-old mussels) were loaded into the mesh tubing or socks (three per station, $n = 3$) and tied at specific intervals on the long ropes and grown until October 2001.

Sample Collection and Transportation. Seawater, mussel (∼30−50), and plankton samples were collected in June, August, September, and October 2000 as well as in April, June, August, September, and October 2001 as described in **Table 1**. Every attempt was made to collect samples within a week range of each other. However, due to difficult weather conditions and/or insufficient logistics it was not possible to follow the above sampling protocol in every sampling month. Plankton were collected using a SEA-GEAR model 900 plankton net (SEA-GEAR Corp., Melbourne, FL). The net dimensions were 3 m long with a 1 m wide mouth opening and a mesh size of 5 μm. The suspended material (10−15 mL) was filtered on a precombusted 47 mm GF/C Whatman glass fiber filter using a Fisher brand filtration apparatus. The glass fiber filter, which contained the plankton material, was used in subsequent analyses. The samples were transported to the laboratory in an insulated Styrofoam container covered with crushed ice.

Table 1. Details of Sampling Days for Mussels and Plankton Obtained from Two Aquaculture Sites (Two Stations per Site), Charles Arm and Fortune Harbor

sampling interval		sampling site			
		Charles Arm		Fortune Harbor	
month	year	station I	station II	station I	station II
June	2000	6	6	29	29
Aug		15	15	10	10
Sept		20	20	13	13
Oct		20	20	13	13
April	2001	7	ND[a]	6	ND
May/June		May 30	May 30	June 3	June 3
Aug		10	10	8	8
Sept		ND	ND	19	19
Oct		3	3	19	19

[a] Not determined.

Physical and Environmental Measurements. Mussel shell size (length in millimeters) was measured using a Traceable Digital Caliper (Fisher Scientific, Nippon, ON). The wet weight of mussels was determined using a Mettler AE 100 analytical balance (Mettler Instruments AG, Zurich, Switzerland). Seawater temperature and salinity were measured using a Sea-Bird SBE25 Sea Logger CTD (Sea-Bird Electronics, Inc., Bellevue, WA) fitted with a WETStar fluorometer (WET Labs, Philomath, OR) for chlorophyll determination as described in the manufacturer's user's manual (*16*).

Sample Preparation for Chemical and Microbiological Analysis. Mussel shells were opened and the shell liquor and meats collected in a beaker and subsequently homogenized over a 60 s period using a commercial Waring blender (Dynamic Corp. of America, New Hartford, CT). All subsequent analyses were performed on this homogenate. Ten grams of the homogenate was mixed with 90 mL of 0.1% peptone water (10 g of peptone and 5 g of NaCl in 1 L of distilled water, pH 7.2−7.3) for a further 30 s. Serial decimal dilutions from this mixture were carried out in 9 mL of 0.1% peptone water. Water samples were collected using Niskin bottles.

Microbiological Analysis of Prepared Samples. Diluted samples (0.5 mL) of the prepared samples were spread with a sterile glass spreader on prepoured marine agar (Becton Dickinson Microbiology Systems, Sparks, MD). Inoculated plates were incubated at 4 °C for 10 days to determine psychrotrophic counts (PPC) and at 30 °C for 48 h to determine aerobic plate counts (APC). Bacterial colonies on the agar were counted using a Quebec counter and recorded as colony-forming units (cfu) per milliliter of seawater or gram of mussel meat.

Chemical Analysis of the Samples: Lipid Extraction and Determination of Fatty Acid Composition and Concentration. Lipids were extracted from blue mussels and/or plankton as described by Budge and Parrish (*17*). Fatty acid methyl esters (FAME) of the lipid extracts were prepared using a 14% BF_3−methanol mixture (*18*). The FAME were analyzed using a Varian model 3400 gas chromatograph (Varian Canada Inc., St. Larent, PQ) with an on-column injector equipped with a 0.25 μm film thickness Omegawax-coated fused silica column (Omegawax 320; 30 m length, 0. 32 mm i.d.; Supelco, Bellefonte, PA). The flow rate of hydrogen (carrier gas) was 2 mL/min, and the flow rates for air and helium were 300 and 30 mL/min, respectively. The oven was programmed to hold at 65 °C for 0.5 min followed by a ramp at 40 °C/min to 195 °C, then hold for 15 min, then ramp at 2 °C/min to 220 °C, and hold for 0.75 min. The flame ionization detector (FID) was isothermal at 260 °C, and the injector temperature was raised from 150 (hold for 0.5 min) to 250 °C at a rate of 200 °C/min. The injector was kept at 250 °C for 10 min. The FAME were identified by comparing their retention times with those of authentic standard

Table 2. Seasonal and Annual Variation in Salinity, Temperature, and Chlorophyll *a*, n-3 Polyunsaturated Fatty Acids, Eicosapentaenoic Acid, and Docosahexaenoic Acid of Seawater at Newfoundland Blue Mussel (*M. edulis*) Aquaculture Sites[a]

month	year	temp (°C)	salinity (psu)	chlorophyll *a* (µg/L)	n-3 PUFA (% of total fatty acids)	EPA (% of total fatty acids)	DHA (% of total fatty acids)
June	2000	7.04 ± 1.5[a]	31.3 ± 0.2[a]	0.48 ± 0.28*	3.2 ± 2.48[a]	0.81 ± 1.2[a]	0.72 ± 0.42[a]
Aug		14.8 ± 1.3[b]	30.4 ± 0.3[ac]	0.97 ± 0.0	43. 63 ± 5.87[b]	14.5 ± 1.8[bc]	19.86 ± 3.1[bc]
Sept		10.7 ± 1.2[c]	29.9 ± 0.6[ac]	1.05 ± 0.0	51.26 ± 5.03[b]	15.1 ± 2.1[bc]	28.05 ± 4.3[c]
Oct		ND	ND	ND	43.20 ± 3.2[b]	13.4 ± 2.0[bc]	22.34 ± 4.2[c]
April	2001	−1.08 ± 0.3[d]	32.2 ± 0.0[b]	0.65 ± 0.2	26.54 ± 1.63[c]	11.7 ± 3.8[b]	8.3 ± 0.9[d]
May/June		1.56 ± 0.7[e]	31.3 ± 1.0[abc]	1.1 ± 0.7	19.46 ± 5.61[c]	9.9 ± 2.4[c]	7.3 ± 2.6[d]
Aug		15.6 ± 0.7[b]	28.97 ± 1.0[c]	0.84 ± 0.3	50.15 ± 6.76[b]	17.5 ± 1.2[b]	27. 0 ± 6.3[c]
Sept		ND	ND	ND	42.28 ± 5.91[b]	14.5 ± 1.1[bc]	19.3 ± 5.0[bc]
Oct		12.0 ± 1.2[c]	29.82 ± 0.5[c]	0.92 ± 0.3	39.19 ± 8.99[b]	13.4 ± 1.5[bc]	19.7 ± 2.0[bc]

[a] Values are means of the four stations ± SD; means with different letters are significantly different ($P < 0.05$) from one another (ANOVA, Tukey test). All data were tested for normality and equal variance. *, no statistical differences ($P > 0.05$) were observed among chlorophyll values, but this is the lowest value in the column. ND, not determined.

Table 3. Seasonal and Annual Variations in the Bacterial Fatty Acid (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) Proportions of Plankton Samples Obtained from Blue Mussels (*M. edulis*) Aquaculture Sites in 2000 and 2001[a]

		% of total fatty acids							
month	year	*i*15:0	*ai*15:0	*i*16:0	*ai*16:0	*i*17:0	*ai*17:0	17:0	total
June	2000	2.34 ± 0.53[a]	1.30 ± 0.42[a]	0.72 ± 0.09[a]	0.13 ± 0.27[a]	1.33 ± 0.71[a]	0.63 ± 0.23[a]	1.35 ± 0.58[a]	10.62 ± 1.98[a]
Aug		0.55 ± 0.06[b]	0.27 ± 0.12[b]	0.28 ± 0.10[b]	0.08 ± 0.16[a]	0.80 ± 0.39[a]	0.42 ± 0.22[a]	1.09 ± 0.20[a]	4.67 ± 0.41[b]
Sept		0.65 ± 0.16[b]	0.26 ± 0.09[bc]	0.21 ± 0.13[b]	0.23 ± 0.14[b]	0.56 ± 0.23[a]	0.22 ± 0.16[ab]	0.88 ± 0.17[a]	3.91 ± 1.14[b]
Oct		1.00 ± 0.30[b]	0.34 ± 0.22[b]	0.31 ± 0.07[b]	0.37 ± 0.23[b]	0.60 ± 0.17[a]	0.13 ± 0.09[b]	0.71 ± 0.29[a]	4.43 ± 1.50[b]
April	2001	0.10 ± 0.14[c]	0.11 ± 0.15[c]	0.00 ± 0.00[c]	0.28 ± 0.06[c]	0.20 ± 0.28[b]	0.16 ± 0.22[b]	0.11 ± 0.15[b]	1.64 ± 0.00[c]
May/June		0.29 ± 0.14[c]	0.21 ± 0.02[b]	0.00 ± 0.00[c]	0.26 ± 0.19[a]	0.45 ± 0.12[ab]	0.35 ± 0.40[ab]	0.41 ± 0.28[ab]	2.66 ± 0.79[c]
Aug		0.56 ± 0.12[b]	0.25 ± 0.14[bc]	0.18 ± 0.07[b]	0.02 ± 0.03[b]	0.63 ± 0.28[a]	0.23 ± 0.08[ab]	0.66 ± 0.22[a]	3.95 ± 0.87[b]
Sept		0.65 ± 0.05[b]	0.47 ± 0.07[d]	0.39 ± 0.11[b]	0.03 ± 0.04[b]	0.79 ± 0.30[a]	0.41 ± 0.12[a]	0.89 ± 0.02[a]	4.77 ± 0.72[b]
Oct		0.65 ± 0.23[b]	0.34 ± 0.07[b]	0.25 ± 0.11[b]	0.00 ± 0.00[c]	0.80 ± 0.13[a]	0.30 ± 0.07[ab]	0.88 ± 0.22[a]	4.41 ± 0.68[b]

[a] Values are means of the four stations ± SD; means with different letters are significantly different ($P < 0.05$) from one another (ANOVA, Tukey test). All data were tested for normality and equal variance.

mixtures (PUFA 1, PUFA 3, Supleco 37 component FAME mixture and bacterial acid methyl ester mixture; Supelco, Canada Ltd., Oakville, ON) and quantified using area normalization and presented as area percent of the total fatty acids. To quantify the essential fatty acid concentrations in the mussels, PUFA proportions and lipid class data were combined (*17*).

Statistical Analysis. All experiments were replicated three times ($n = 3$). Mean ± standard deviation (SD) was reported for each case. Prior to statistical analysis, results of microbiological analysis were transformed to log values. Analyses of variance (ANOVA) and Tukey's studentized test were performed at a level of $P < 0.05$ to evaluate significance of differences between mean values (*19*).

RESULTS

Seasonal and Annual Variation in Environmental Characteristics at Blue Mussel Aquaculture Sites. Water temperature increased from a minimum of −1.08 °C in April 2001 (Fortune Harbor I) to a maximum of 15.6 °C in August 2001 (Charles Arm II). Water salinity fluctuated within the four stations from a minimum of 28.1 practical salinity units (psu) in August 2001 (Fortune Harbor II) to a maximum of 32.2 psu in April 2001 (Fortune Harbor I). The concentration of chlorophyll *a* ranged from 0.48 µg/L in June 2000 (Charles Arm I) to 1.1 µg/L in May/June 2001 (Fortune Harbor I). The values for the four stations were averaged and tabled against sampling month to obtain seasonal and annual variations in environmental variables in 2000 and 2001 (**Table 2**). Initial temperature values in June 2000 were low; thereafter, they increased in September 2000 and then decreased to the lowest values in April 2001 followed by a gradual increase to reach high values in August 2001. The reverse trend was observed for salinity (psu), which was high in June 2000, then decreased in September and October 2000 followed by a sharp increase in April 2001, then decreased again from August to October 2001. No significant seasonal or annual variation in chlorophyll *a* was observed ($P > 0.05$), although high values were evident in September and October 2000 and May/June 2001 (**Table 2**).

There were no significant differences ($P > 0.05$) in APC and PPC of blue mussels and water samples within the four stations throughout the sampling period in years 2000 and 2001, with some exceptions (data not shown). The highest APC of mussels was in August 2000 (3.41 ± 0.05 cfu/g) and August 2001 (3.61 ± 0.06 cfu/g). These values corresponded to the lowest PPC (2.86 ± 0.06 cfu/g) and (3.07 ± 0.34 cfu/g) in August 2000 and 2001, respectively. A similar trend was observed when water samples were considered (data not shown). The highest APC of water samples was in August 2000 (3.49 ± 0.07 cfu/g) and August 2001 (3.53 ± 0.07 cfu/g), which corresponded to PPC values of 2.92 ± 0.10 cfu/g in August 2000 and 3.20 ± 0.07 cfu/g in August 2001.

No significant differences ($P > 0.05$) in total n-3 PUFA, DHA, and EPA or in total and individual bacterial fatty acid composition (percent of total fatty acids) of plankton samples among the four stations were observed throughout the sampling period in 2000 and 2001 (data not shown). Therefore, the fatty acid composition in the four stations was averaged to give the seasonal variations in fatty acid compositions at mussel culture sites (**Tables 2** and **3**). Total n-3 PUFA was the lowest in June 2000 followed by May/June 2001 and the highest in August to September in both 2000 and 2001. DHA proportions were initially low (0.72 ± 0.42%) in June 2000 but increased

Table 4. Seasonal and Annual Variation in Shell Size, Meat Content, n-3 Polyunsaturated, Eicosapentaenoic Acid, and Docosahexaenoic Acid in the Cultivated Mussels[a]

month	year	size (mm)	meat content (g)	n-3 PUFA (% of total fatty acids)**	EPA (% of total fatty acids)	EPA (mg/g of wet wt)	DHA (%)	DHA (mg/g of wet wt)
June	2000	23.50 ± 0.23[a]	0.48 ± 0.28[a]	47.13 ± 5.5	19.52 ± 3.2[a]	2.86 ± 1.06[a]	17. 03 ± 3.7[a]	2.23 ± 0.36[a]
Aug		30.43 ± 1.78[b]	0.66 ± 0.0[a]	44.07 ± 11.1	12.00 ± 1.9[b]	1.03 ± 0.10[ab]	22.25 ± 3.9[b]	1.80 ± 0.19[ab]
Sept		37.93 ± 4.73[cd]	1.13 ± 0.5[ab]	53.12 ± 0.9	12.25 ± 1.3[b]	0.78 ± 0.16[b]	25.14 ± 1.3[b]	1.64 ± 0.23[ab]
Oct		43.78 ± 1.78[d]	2.06 ± 0.4[b]	53.97 ± 1.53	13.22 ± 1.4[b]	1.10 ± 0.13[ab]	25.00 ± 1.5[b]	2.07 ± 0.55[ab]
April	2001	50.85 ± 4.45[d]	2.66 ± 0.1[bc]	50.73 ± 1.58	19.56 ± 2.5[a]	0.95 ± 0.14[ab]	19.92 ± 4.60[b]	1.29 ± 0.09[b]
May/June		52.18 ± 3.44[d]	3.43 ± 0.59[c]	45.67 ± 4.0	21.40 ± 1.8[a]	NA	14.32 ± 3.2[a]	NA
Aug		59.35 ± 1.08*	3.62 ± 0.25[c]	47.63 ± 2.8	15.25 ± 1.6[b]	NA	23.49 ± 1.3[b]	NA
Sept		53.65 ± 2.33[d]	3.84 ± 0.88[c]	47.01 ± 1.5	13.2 ± 1.7[b]	NA	22.35 ± 0.0[b]	NA
Oct		50.83 ± 4.52[d]	3.69 ± 0.96[cb]	49.17 ± 1.2	13.5 ± 1.5[b]	NA	23.69 ± 0.5[b]	NA

[a] Values are means of the four stations ± SD; means with different letters are significantly different ($P < 0.05$) from one another (ANOVA, Tukey test). All data were tested for normality and equal variance. When these tests failed, Dunn's method was used. *, this was value is an anomaly. **, no statistical differences ($P > 0.05$) were observed among n-3 PUFA values. NA, not available.

Table 5. Seasonal and Annual Variations in the Bacterial Fatty Acids (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) Content of Cultivated Mussels in 2000 and 2001[a]

month	year	% of total fatty acids							
		*i*15:0	*ai*15:0	*i*16:0	*ai*16:0	*i*17:0	*ai*17:0	17:0	total
June	2000	0.20 ± 0.29[a]	0.06 ± 0.08[a]	0.22 ± 0.31[a]	0.44 ± 0.07[a]	0.80 ± 0.42	0.66 ± 0.10[a]	0.64 ± 0.33[a]	3.60 ± 1.68
Aug		0.13 ± 0.06[a]	0.14 ± 0.28[a]	0.24 ± 0.08[a]	0.49 ± 0.04[a]	1.20 ± 0.34	0.70 ± 0.52[a]	1.03 ± 0.11[a]	4.62 ± 0.67[ab]
Sept		0.10 ± 0.08[a]	0.05 ± 0.08[a]	0.25 ± 0.08[a]	0.86 ± 0.11[b]	1.18 ± 0.28	1.20 ± 0.10[b]	1.14 ± 0.25[a]	5.50 ± 0.71[a]
Oct		0.10 ± 0.07[a]	0.03 ± 0.04[a]	0.20 ± 0.08[a]	0.72 ± 0.40[b]	0.97 ± 0.21	0.86 ± 0.41[b]	0.71 ± 0.22[a]	4.19 ± 1.39[ab]
April	2001	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.84 ± 0.32[b]	0.13 ± 0.19[c]	1.16 ± 0.36	0.24 ± 0.24[c]	0.31 ± 0.26[b]	3.13 ± 0.82[ab]
May/June		0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.11 ± 0.07[c]	0.34 ± 0.25[a]	0.75 ± 0.19*	0.15 ± 0.18[c]	0.56 ± 0.13[ab]	2.30 ± 0.52[b]
Aug		0.04 ± 0.07[a]	0.05 ± 0.05[a]	0.15 ± 0.05[c]	0.40 ± 0.07[a]	0.76 ± 0.17	0.33 ± 0.03[c]	0.99 ± 0.14[a]	3.36 ± 0.37[ab]
Sept		0.10 ± 0.08[a]	0.08 ± 0.03[a]	0.20 ± 0.13[a]	0.53 ± 0.01[a]	1.03 ± 0.17	1.01 ± 0.22[b]	1.16 ± 0.23[a]	4.70 ± 1.67[ab]
Oct		0.08 ± 0.08[a]	0.08 ± 0.05[a]	0.27 ± 0.05[a]	0.60 ± 0.08[a]	0.97 ± 0.34	0.63 ± 0.52[bc]	1.00 ± 0.25[a]	3.60 ± 2.45[ab]

[a] Values are means of the four stations ± SD; means with different letters are significantly different ($P < 0.05$) from one another (ANOVA, Tukey test). All data were tested for normality and equal variance. *, no statistical differences ($P > 0.05$) were observed among *i*17:0 values, but this is the lowest value in the column.

gradually to reach 28.05 ± 4.3% in September 2000. A similar trend was observed in 2001. DHA proportion in plankton in May/June 2001 was 7.3 ± 2.6% but increased gradually to 19.7 ± 2.0% in October 2001. EPA content in June 2000 was low at 0.8 ± 1.2% in June 2000 and at 9.9 ± 2.4% in May/June 2001 but increased in August and September 2000 and 2001 followed by a decrease in October of both years. The total and individual bacterial fatty acids (*i*15:0, *ai*15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, and 17:0) of algae were lowest in April 2000 and highest in June 2000. There was no significant difference ($P > 0.05$) in bacterial fatty acids in plankton samples from August to October 2000 or from August to October 2001 (**Table 3**).

Seasonal and Annual Variations in Quality Attributes of Cultivated Mussels. There were no significant differences ($P > 0.05$) in shell length among mussels from the four stations throughout the sampling period. The average lengths from all sites and stations were tabled against the sampling months (**Table 4**). There was a gradual linear increase in the average shell length of mussels from June 2000 (23.50 ± 0.23 mm) until April 2001 (50.85 ± 4.45 mm), when a plateau was reached. Thereafter, there were no significant differences ($P > 0.05$) in the average shell lengths of the mussels (April−October 2001), with one exception in August 2001. The average shell length of mussels collected in August 2001 was significantly higher ($P < 0.05$) than those of other sampling dates. There was no significant difference ($P > 0.05$) in the meat content among the mussels obtained from the four stations throughout the sampling period in 2000 and 2001. The mussel weights were averaged from the four stations and tabled against the sampling intervals (**Table 4**). There was a gradual linear increase in the meat content of mussels from 0.48 ± 0.28 g in June 2000 to 3.69 ± 0.96 g in October 2001. However, the linear growth reached a plateau in May/June 2001, after which there were no significant ($P > 0.05$) differences in average weights of harvested blue mussels.

No significant differences ($P > 0.05$) were observed in total n-3 PUFA, DHA, EPA, or total and individual bacterial fatty acids (percent of total fatty acids) in mussels from the four stations throughout the sampling period in 2000 and 2001 (data not shown). To obtain seasonal variations in the above fatty acids during the growth of mussels in 2000 and 2001, values for the four stations were averaged as shown in **Tables 4** and **5**. The average n-3 PUFA content throughout the cultivation period was ~50% of the total fatty acids. However, EPA content was initially high at 19.5 ± 3.2% of total fatty acids in June 2000 and then decreased to 13.2 ± 1.4% in October 2000. This trend was repeated in 2001 with mussels having higher ($P < 0.05$) proportions of EPA in April and May/June than in August to October. Meanwhile, DHA was initially low at 17.03 ± 3.7% in June 2000 and thereafter increased to 25.00 ± 1.5% in October 2000. A similar trend was observed in 2001, where the DHA content was lowest in April and May/June (14.32 ± 3.2%) and increased to 23.69 ± 3.50% in October. The initial total bacterial fatty acids were 3.60 ± 1.68% in June 2000, which increased afterward from August to October 2000. The average total bacterial fatty acids detected in blue mussels in May/June 2001 were 2.30 ± 0.52%, followed by an increase from August to October 2001 (**Table 5**). Bacterial fatty acids (*i*15:0 and *ai*15:0) were highest in June−August 2000 and lowest in April−June 2001; *i*16:0 was highest in April 2001 and lowest

in May/June 2001; *ai*16:0 was highest in September–October 2000 and lowest in April 2001; and *ai*17:0 and 17:0 were lowest in April 2001, whereas no significant differences ($P > 0.05$) were observed in *i*17:0 throughout the cultivation period (**Table 5**).

DISCUSSION

No seasonal or annual variation in chlorophyll *a* was observed, although two high values were evident in September and October 2000 and May/June 2001. This can be attributed to the large standard deviation, which indicates that some stations may have had more chlorophyll at the time of sampling. Therefore, to obtain meaningful data for chlorophyll *a* content for the selection of future aquaculture sites, more frequent measurements at different tidal cycles may be required (*6*).

Seasonal and annual temperature fluctuation of the ocean water in the four stations affected their salinity, microbial content, and algal fatty acid compositions, which in turn affected the quality attributes of cultivated mussels. Similar observations were reported previously (*5*, *11*). Therefore, temperature measurement can be used as one of the environmental variables to aid in the selection of the future mussel aquaculture sites.

The higher salinity values in April 2001 can be explained by low temperatures, which lead to the formation of ice and reduced runoff because of water being trapped in ice and snow. Once the ice started to melt, runoff of rain and river water started to dilute the aquaculture sites during June–August, and salinity decreased. This fluctuation in salinity may not adversely affect the growth or biochemical composition of mussels as they can tolerate salinity from 25 to 33 psu (*3*).

High temperatures in August of 2000 and 2001 may explain the relatively high APC but low PPC in mussels and water. This indicates that the microbial content of water is directly affecting the microbial content of mussels. However, the bacterial counts of water at the examined aquaculture sites were lower than some published values for marine environments (*20*), and this perhaps can be explained by the relatively low temperatures observed in this study with a maximum of 15.6 °C in the summer of 2000. Various maximum acceptable levels of APC (10^5–10^7) in bivalves have been set, but it is generally recommended that APC should not exceed 10^5 or log 5 cfu/g (*21*). Because the cultivated mussels examined in this study never reached this maximum limit, they can be harvested throughout the year.

The dominance of algal fatty acids by DHA at the aquaculture sites from August to October in 2000 and 2001 can be explained by the presence of dinoflagellates in which DHA is the major fatty acid (*18*, *22*). Furthermore, the higher proportions of EPA in August 2000 and 2001 can be attributed to the presence of diatoms, in which EPA is the dominant fatty acid (*22*–*24*). Both DHA and EPA were low in April 2001, indicating a lower algae population due to subzero temperatures (*6*) or inappropriate light conditions due to long nights and snow and ice coverage of the aquaculture sites. The fatty acid composition of algae directly affected the fatty acid profiles of cultivated mussels in various seasons and years. The filter-feeding behavior of bivalves and the algal concentrations in the aquaculture sites (*25*) can explain this effect. Furthermore, EPA and DHA content in cultured mussels showed opposite trends in the spring and summer, indicating that when one is increasing, the other one would decrease. This may be explained by temperature changes in the spring to subfreezing values, which led to an increase in the production of EPA in bivalves (*26*, *27*). On the basis of fatty acid proportions in cultured mussels in this study, the optimum harvest time may be April–June for EPA-rich mussels and August–October for DHA-rich mussels. However, in terms of concentrations, June may be the best month for both essential fatty acids. The relatively higher values for total bacterial fatty acids in plankton and mussel samples in the summer and fall of 2000 and 2001 compared with spring 2001 values may be attributed to contamination with runoff waters and spawning of mussels. It is also possible that mussels are consuming bacteria associated with algae (*3*, *28*). The use of bacterial fatty acids as rapid indicators of plankton composition in the aquaculture sites and quality of cultivated mussels can be further explored.

The optimum growth temperatures of mussels are between 10 and 20 °C, explaining the rapid growth (in shell length and meat weight) of mussels from June to October 2000, after which growth slowed from October to April 2001. Furthermore, mussels reached market size by May/June 2001 with no further significant growth in shell length or meat weight. The high value of shell size in August 2001 is considered to be an anomaly, perhaps due to the collection of mussels with relatively longer shells. Therefore, the optimum growth period in terms of meat content and shell size may be achieved within 1 year of cultivation.

ABBREVIATIONS USED

ANOVA, analysis of variance; BFA, bacterial fatty acids; cfu, colony-forming units; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; psu, practical salinity units; PUFA, polyunsaturated fatty acids; UHP, ultrahigh purity.

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LITERATURE CITED

(1) McDonald, C.; Gallant, R.; Couturier, C. Canadian mussel aquaculture: an industry with room to grow. *Bull. Aquat. Assoc. Can.* **2002**, *102*–*103*, 87–92.
(2) McLeod, D. The life and times of the "*Myti*" mussels. *Bull. Aquat. Assoc. Can.* **2002**, *102*–*103*, 8–16.
(3) Sutterlin, A.; Agget, D.; Courturier, C.; Scaplen, R.; Idler, D. Mussel culture in Newfoundland waters. *Marine Science Research Laboratory Technical Report 23*; Memorial University of Newfoundland (MUN): St. John's, NL, Canada, 1981.
(4) Parrish, C. C.; McKenzie, C. H.; McDonald, B. A.; Hatfield, E. A. Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove, Newfoundland. *Mar. Ecol. Prog. Ser.* **1995**, *129*, 151–164.
(5) Stirling, H. P.; Okumus, I. Growth and production of mussels (*Mytilus edulis* L) suspended at salmon cages and shellfish farms in two Scottish sea lochs. *Aquaculture* **1995**, *134*, 193–201.
(6) Ogilive, S. C.; Ross, A. H.; Schiel, D. R. Phytoplankton biomass associated with mussel farms in Beatrix Bay, New Zealand. *Aquaculture* **2000**, *181,* 71–80.
(7) Slabyj, B. M.; Creamer, D. L.; True, R. H. Seasonal effect on yield, proximate composition and quality of blue mussels (*Mytilus edulis*) meats obtained from cultivated and natural stocks. *Mar. Fish. Rev.* **1977**, *40*, 18–23.
(8) Kryznowek, J.; Wiggin, K. Seasonal variation and frozen storage stability of blue mussels (*Mytilus edulis*). *J. Food Sci.* **1979**, *44,* 1644–1648.

(9) Krzynowek, K. Sterols and fatty acids in seafood. *Food Technol.* **1985**, *39* (2), 61−68.
(10) Dolmer, P. Seasonal and spatial variability in growth of (*Mytilus edulis* L.) in a brackish sound: comparison of individual mussel growth and growth of size classes. *Fish. Res.* **1998**, *34*, 17−26.
(11) Orban, E.; Di Lena, G.; Nevigato, T.; Casini, I.; Marzetti, A.; Caproni, R. Seasonal changes in meat content, condition index and chemical composition of mussels cultured in two different Italian sites. *Food Chem.* **2001**, *77,* 57−65.
(12) Newton, I. S. Long chain fatty acids in health and nutrition. In *Seafood in Health and Nutrition, Transformation in Fisheries and Aquaculture: Global Perspectives*; Shahidi, F., Ed.; ScienceTech Publishing: St. John's, NL, Canada, 2000; pp 15−28.
(13) Uauy, R., Mena, P.; Rojas, C. Essential fatty acids in early life: structure and functional role. *Proc. Nutr. Soc.* **2000**, *59*, 3−15.
(14) Mastronicolis, S. K.; German, J. B.; Smith, G. M. Isolation and fatty acid analysis of neutral and polar lipids of the food bacterium *Listeria monocytogenes*. *Food Chem.* **1996**, *57,* 451−456.
(15) Osipov, G. A.; Turova, E. S. Studying species composition of microbial communities with the use of gas chromatography−mass spectrometry. *FEMS Microbiol. Rev.* **1997**, *20*, 437−446.
(16) WETStar fluorometers in http://www.wetlabs.com/products/eflcombo/fl.htm, accessed on Dec 29, 2005.
(17) Budge, S. M.; Parrish, C. C. FA determination in cold water marine samples. *Lipids* **2003**, *38*, 781−791.
(18) Copeman, L. A.; Parrish, C. C. Marine lipids in a cold coastal system: Gilbert Bay, Labrador. *Mar. Biol.* **2003**, *143*, 1213−1227.
(19) Freund, J. H.; Williams, F. J.; Perles, B. M. Decision making correlation. In *Elementary Business Statistics*, *The Modern Approach*, 5th ed.; Prentice Hall: Englewood Cliffs, NJ, 1988; pp 494−520.
(20) Lizarraga-Partida, M. L.; Anguiano-Beltran, C.; Searcy-Bernal, R.; Vazquez-Moreno, E. Bacterial water quality in Abalone farms of Baja California. *J. Shellfish Res.* **1998**, *17*, 689−692.
(21) Anonymous. Microbiological criteria for raw molluscan shellfish. *J. Food Prot.* **1992**, *55*, 667−671.
(22) Budge, S. M.; Parrish, C. C.; McKenzie, C. H. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Mar. Chem.* **2001**, *76*, 285−303.
(23) Ramos, C. S.; Parrish, C. C.; Quibuyen, T. A.; Abrajano, T. A. Molecular and carbon isotopic variations in lipids in rapidly settling particles during a spring phytoplankton boom. *Org. Geochem.* **2000***, 34,* 195−207.
(24) Parrish, C. C.; Wells, J. S.; Yang, Z.; Dabinett, P. Growth and lipid composition of scallop juveniles, *Placopecten magellanicus*, fed the flagellate *Isochrysis galbana* with varying lipid composition and diatom *Chaetoceros muelleri. Mar. Biol*. **1998**, *133*, 461−471.
(25) Riisgard, H. U. Filtration rate and growth in the blue mussels *Mytilus edulis linneaus,* 1758: dependence on algal concentration. *J. Shellfish Res.* **1991**, *10*, 29−35.
(26) Hall, J. M.; Parrish, C. C.; Thompson, R. J. Importance of unsaturated fatty acids in regulating bivalve and finfish membrane fluidity ion response to changes in environmental temperatures. In *Seafood in Health and Nutrition, Transformation in Fisheries and Aquaculture: Global Perspectives*; Shahidi, F., Ed.; ScienceTech Publishing: St. John's, NL, Canada, 2000; pp 521−546.
(27) Hall, J. M.; Parrish, C. C.; Thompson, R. J. Eicosapentaenoic acid regulates scallop (*Placopecten magellanicas*) membrane fluidity in response to cold temperature. *Biol. Bull.* **2002**, *202*, 201−203.
(28) Rheinheimer, G. Interrelationship between bacteria and phytoplankton in a marine area. *Bacteriol. Mar.* **1982**, *17*, 101−106.

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