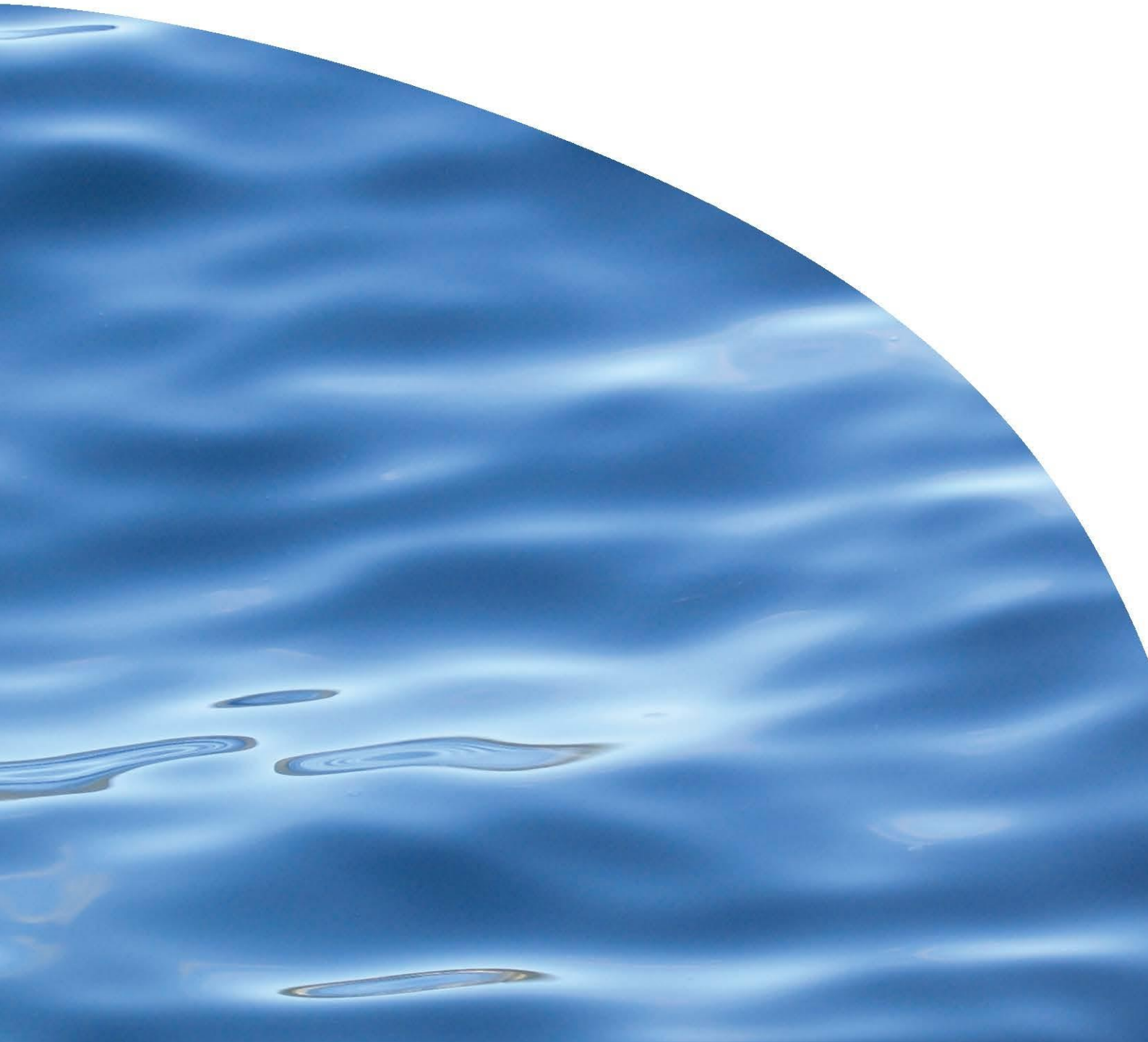
CAWTHRON INSTITUTE

REPORT NO.2532

LIPID AND FATTY ACID COMPOSITION OF NEW ZEALAND GREENSHELL™ MUSSELS (GSM) FROM THREE FARMING SITES



LIPID AND FATTY ACID COMPOSITION OF NEW ZEALAND GREENSHELL™ MUSSELS (GSM) FROM THREE FARMING SITES

DONATO ROMANAZZI

Prepared for Bay of Connections

CAWTHRON INSTITUTE
98 Halifax Street East, Nelson 7010 | Private Bag 2, Nelson 7042 | New Zealand
Ph. +64 3 548 2319 | Fax. +64 3 546 9464
www.cawthron.org.nz

REVIEWED BY:
Darby Brooke

APPROVED FOR RELEASE BY:
Paul McNabb

ISSUE DATE: 26 May 2014

RECOMMENDED CITATION: D. Romanazzi 2014. Lipid and fatty acids composition of New Zealand Green Shell Mussels (GSM) from three farming sites. Prepared for Bay of Connections. Cawthron Report No.2532. 9 p.

1. SCOPE OF STUDY

This report summarizes the analysis of samples of greenshell™ mussels (GSM) collected from 3 different aquaculture regions in Marlborough, Coromandel and the Bay of Plenty. The focus of this study was the lipid class profile, fatty acid (FA) profile and phospholipids (PL) content of the mussels collected from the different regions. A brief literature review on the possible health benefits of GSM lipids has also been included.

2. ANALYTICAL RESULTS

Samples of greenshell™ mussels (GSM) were collected from different sites in the Marlborough Sounds, Coromandel and Bay of Plenty. Twenty mussels from each of the different sites within these three geographical areas were blended together to give one representative sample for each area. Samples from the Marlborough Sounds and Coromandel farming areas were kindly provided by the Marlborough Shellfish Quality Programme (MSQP) and the Coromandel Marine Farming Association (CMFA) respectively, and were collected between the 17th and 21st of March 2014. Bay of Plenty samples were collected between the 5th and 7th of May 2014 from an offshore mussel farm.

Live mussels from each region were drained and homogenized, and homogenates were analysed for their total fat content, lipid classes, and profiles of fatty acids and phospholipids.

2.1. Lipid classes

Samples of GSM homogenate were extracted following a modified Bligh-Dyer protocol. A single-phase extraction with $CHCl_3$:MeOH yielded the total lipid extract (TLE).
Lipid classes were analysed with an Iatroscan MKV thin-layer chromatography-flame ionization detector (TLC-FID) analyser (Iatron Laboratories, Japan).
Samples were spotted onto silica gel SIII Chromarods (5 µm particle size) and developed in a glass tank lined with pre-soaked filter paper. Two solvent systems were used for the lipid separation: hexane:diethyl ether:acetic acid (60:17:0.1, v/v/v) and hexane:diethyl ether (70:30, v/v). After development for 25 min, the Chromarods were oven-dried and then immediately analysed. The FID was calibrated for each compound class with phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, hydrocarbon (squalene), wax ester (WE),(derived from fish oil), triacylglycerol (TAG, from fish oil) and diacylglyceryl ether (DAGE, purified from shark liver oil). Results obtained are reported in Table 1 as % of the total lipids.

Table1: Lipid classes profile

	Lipid class %			
Sample	***TAG***	***CE***	***ST***	***PL***
Marlborough	37.3	15.5	9.3	37.9
Coromandel	37.5	4.8	13.7	44.2
Bay of Plenty	25.3	5.8	15.7	53.3

TAG: Triacylglycerols; CE: cholesterol esters; ST: Sterols; PL: Phospholipids

2.2. Fatty Acids profile

An aliquot of the TLE from each sample type was trans-methylated, as described in the AOAC 963.22 OMA, to obtain fatty acid methyl esters (FAME). Samples were then analysed by gas chromatography (GC) with FID detection, and fatty acids were quantified using a SUPELCO® 37 component FAME mix as a reference standard. Results for total fat content and fatty acid profiles of samples are summarized in Tables 2 and 3, respectively.

Table2: Total fat content

Sample	***Total lipids % (g/100g sample)***
Marlborough	1.3
Coromandel	1.4
Bay of Plenty	0.6

Table 3: Fatty acid profiles

	Sample			
Fatty Acid	***Marlborough***	***Coromandel***	***Bay of Plenty***	*Units*
Saturated Fat	0.4	0.4	0.2	g/100g
Monounsaturated Fat	0.2	0.2	0.1	g/100g
Polyunsaturated Fat	0.6	0.6	0.3	g/100g
Omega 3 Fatty Acids	0.5	0.5	0.2	g/100g
Omega 6 Fatty Acids	0.1	0.1	<0.1	g/100g
Omega 9 Fatty Acids	<0.1	<0.1	<0.1	g/100g
EPA	0.22	0.18	<0.1	g/100g
DHA	0.20	0.28	0.12	g/100g
C4:0 butyric acid	<0.1	<0.1	<0.1	% fatty acids
C6:0 caproic acid	<0.1	<0.1	<0.1	% fatty acids
C8:0 caprylic acid	<0.1	<0.1	<0.1	% fatty acids
C10:0 capric acid	<0.1	<0.1	<0.1	% fatty acids
C11:0 undecanoic acid	<0.1	<0.1	<0.1	% fatty acids
C12:0 lauric acid	<0.1	<0.1	<0.1	% fatty acids
C13:0 tridecanoic acid	<0.1	<0.1	<0.1	% fatty acids
C14:0 myristic acid	5.6	5.0	1.9	% fatty acids
C14:1 myristoleic acid	<0.1	<0.1	<0.1	% fatty acids
C15:0 pentadecanoic acid	0.8	1.0	0.9	% fatty acids
C15:1 cis-10-pentadecanoic acid	<0.1	<0.1	<0.1	% fatty acids
C16:0 palmitic acid	16.3	16.9	14.8	% fatty acids
C16:1 palmitoleic acid	7.1	5.0	3.6	% fatty acids
C16:2n4	0.5	0.3	<0.1	% fatty acids
C17:0 heptadecanoic acid	1.0	1.2	1.1	% fatty acids
C17:1 cis-10-heptadecanoic acid	<0.1	<0.1	<0.1	% fatty acids
C18:0 stearic acid	4.8	5.0	5.5	% fatty acids
C18:1n7 vaccenic acid	2.8	2.0	1.8	% fatty acids
C18:1n9c oleic acid	1.1	1.5	1.5	% fatty acids
C18:1t	0.2	<0.1	<0.1	% fatty acids
C18:2n6c linoleic acid	2.2	2.5	2.8	% fatty acids
C18:2t	<0.1	<0.1	<0.1	% fatty acids
C18:3n6 alpha linolenic (ALA)	1.5	1.6	1.3	% fatty acids
C18:3n6 gamma linolenic (GLA)	0.2	0.7	<0.1	% fatty acids
C18:3n4	0.4	<0.1	0.9	% fatty acids
C18:4n3 stearidonic acid (SDA)	2.5	2.3	1.5	% fatty acids
C20:0 arachidic acid	0.1	<0.1	<0.1	% fatty acids
C20:1 gadoleic acid	2.5	2.5	3.1	% fatty acids
C20:2 eicosadienoic acid	<0.1	<0.1	<0.1	% fatty acids
C20:3n3 cis-11, 14, 17-eicosatrienoic acid	<0.1	<0.1	<0.1	% fatty acids
C20:3n6 cis-8, 11, 14-eicosatrienoic acid	0.3	0.3	<0.1	% fatty acids
C20:4n3 eicosatetraenoic acid	0.2	0.3	<0.1	% fatty acids
C20:4n6 arachidonic acid (AA)	2.1	2.1	2.7	% fatty acids
C20:5n3 eicosapentaenoic acid (EPA)	16.9	12.5	11.2	% fatty acids
C21:0 heneicosanoic acid	<0.1	<0.1	4.3	% fatty acids
C22:0 behenic acid	<0.1	<0.1	<0.1	% fatty acids
C22:1n9 cetoleic/erucic acid	<0.1	<0.1	<0.1	% fatty acids
C22:2 dicosadienoic acid	<0.1	<0.1	<0.1	% fatty acids
C22:5n3 docosapentaenoic acid (DPA)	1.2	1.2	1.5	% fatty acids
C22:6n3 docosahexaenoic acid (DHA)	15.1	19.8	19.8	% fatty acids
C23:0 tricosanoic acid	<0.1	<0.1	<0.1	% fatty acids
C24:0 lignoceric acid	<0.1	<0.1	<0.1	% fatty acids
C24:1 nervonic acid	<0.1	<0.1	<0.1	% fatty acids

2.3. Phospholipids

The phospholipid (PL) fraction was isolated from each TLE by solid-phase extraction (SPE). An amount of TLE was applied to a silica SPE cartridge, and the PLs-containing fraction was eluted with a $CHCl_3$:MeOH:water mixture
Analysis of the PL-containing fraction was conducted on a Waters Acquity i-Class UPLC equipped with ELS detector. PL families were separated by normal phase chromatography with gradient elution on a BEH HILIC 1.7µm, 2.1 x 100 mm analytical column. The main PLs quantified were phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). PL families were identified by comparison of retention times with those of authentic external standards of PC, PE, and PI purchased from Sigma-Aldrich. Table 4 (below) summarizes the results obtained.

Table 4: PL content

	mg/g of sample		
Sample	***Phosphatidyl Choline (PC)***	***Phosphatidyl Ethanolamine (PE)***	***Phosphatidyl Inositol (PI)***
Marlborough	0.28	0.41	0.04
Coromandel	0.44	1.10	0.08
Bay of Plenty	0.39	0.78	0.06

It should be noted that other forms of PLs are generally present in GSM, however their quantitation and identification was not possible with the methodology utilized in this study. It is likely that the additional unidentified peaks which were observed in chromatograms represent these other PLs, and further work should enable their unequivocal identification.

3. DISCUSSION

This study focused on the differences in lipid composition in GSM collected from three different farming areas around New Zealand.
The results obtained show some minor differences in total lipid content between the samples. In particular, the total lipid content of mussels harvested from the Marlborough Sounds and Coromandel areas is in line with previous data (Murphy *et al*., Nichols *et al*., McLean *et al*.), whilst that of mussels collected in the Bay of Plenty was lower. The latter result was also reflected in a corresponding lower concentration of omega-3 and omega-6 fatty acids per sample wet weight.
No major differences were noticed in the fatty acid (FA) profiles of samples. In samples from all three geographical areas, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accounted for approximately 30% of the total FA, with palmitic acid (C16:0) accounting for another 15% in all 3 samples.

Interestingly, significant differences were noticed in the lipid class profiles of samples from each area, as shown in Table 1. Mussels collected in the Marlborough region had a lower level of PL (37.9%), compared with levels of 44.2% and 53.3% in Coromandel and Bay of Plenty, respectively. This was also confirmed by individual PL analysis performed by UPLC-ELSD, as shown in Table 4. Also, sterol (ST) levels were lower in Marlborough, while those of cholesterol esters (CE) were threefold higher than in the other regions. Overall, despite the lower total lipid content of the Bay of Plenty mussels, over 50% of these total lipids were PL.

These differences in the lipid profiles of the representative samples from the three areas are challenging to explain. However, geographical location is thought to influence lipid content and lipid classes of mussel species, given that variation in the phytoplankton, dinoflagellate and zooplankton populations in the farming areas could conceivably influence the lipid composition of sedentary, filter-feeding molluscs such as mussels.

Other factors which may affect lipid-class profiles of mussels were described by Jeffs *et al*., who reported that GSM inhabit waters of different depths and temperatures, which may affect their basal metabolic profile, in particular, a cooler habitat or climate could result in mussels in such areas having higher levels of storage lipids like TAGs, instead of PLs.

Recently, Miller *et al*. investigated the changes in the lipid class and FA profiles in various organs of male and female GSM. Murphy *et al*. also suggested that the life-cycle of the mussel, particularly the development of the gonads, may affect total lipid composition. The sex of the mussels in this study was not determined, so this remains as another possible cause of the observed variation in lipid class and FA profiles in the samples.

Further research is also required to investigate the effect of different harvesting and processing techniques on the lipid composition and nutritive value of GSM used for human consumption. A greater understanding of the effects of these (and other factors mentioned above) on the lipid profile and quality of GSM will be useful for optimizing both the harvest of GSM, and the processes utilized for their lipid-extraction.

4. HEALTH BENEFITS OF GSM

This study was not intended to demonstrate or verify any health claim associated with GSM consumption. Only a brief literature review has been conducted on health benefits associated with GSM oil and shellfish consumption.

In several studies (James *et al*., Nestel *et al*., Lorenz *et al*., Whitehouse *et al*., Rainsford *et al*.) omega-3 polyunsaturated fatty acids (PUFAs) from marine sources have been associated with reducing risk-factors for cardiovascular disease, such as lowering plasma TAG, reducing potential thromboses and alleviating the symptoms of inflammation. The majority of marine species are rich sources of these long-chain

PUFAs, particularly EPA and DHA. Greenshell™ mussels (GSM) in particular are a good source of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), and farmed GSM are considered to be a sustainable source of LC-PUFA (Miller *et al*.). GSM are generally sold as food, but are also extracted to produce high-value nutraceuticals and dietary supplements, for example Lyprinol® and Seatone®. Several studies have demonstrated the health benefits of GSM lipids (Whitehouse *et al*., Gruenwald *et al*., Treschow *et al*., McPhee *et al*.).
The level of omega-3 PUFA is higher in GSM than in other fish (Miller *et al*.) and therefore they are considered one of the most suitable sources of omega-3 oil for the continuously expanding market for marine oil supplements.

5. REFERENCES

- K.J. Murphy, B.D. Mooney, N.J. Mann, P.D. Nichols, and A.J. Sinclair. *Lipids*, Vol. 37, no. 6 (2002)
- K.J. Murphy, N.J. Mann, and A.J. Sinclair *Asia Pacific J Clin Nutr* 2003; 12(1): 50-60
- Jeffs AG, Holland RC, Hooker SH, Hayden BJ. *J Shellfish Res* 1999; 18; 347-360
- Nichols PD, Virtue P., Mooney BD, Elliott NG, Yearsley GK. FRDC Project 95/122. CSIRO Publication, Hobart, Australia 1998
- MR Miller, L Pearce, BI Bettjeman. *Nutrients* 2014, 6, 1454-1474
- Whitehouse MW, Macrides TA, Kalafatis N, Betts WH, Haynes DR, Broadbent J. *Inflammopharmacology* 1997, 5, 237-246
- Nestel PJ. *Am. J. Clin. Nutr.* 71, 228-231, (2000)
- James MJ, Cleland LG, Gibson RA. *Am. J. Clin. Nutr.* 71, 343-348, (2000)
- Lorenz R., Weber PC., Szimnau P., Heldwein W., Strasser T., Loeshke K. *J. Intern. Med.* 225, 225-232, (1989)
- Rainsford KD, Whitehouse MW. *Drug Res.* 30, 2128-2132, (1980)
- Gruenwald J., Graubaum HJ., Hansen K., Grube B. *Adv. Ther*. 2004, 21, 197-201
- Treschow AP, Hodges LD, Wright PFA, Wynne PM, Kalafatis N, Macrides TN. *Comp. Biochem. Physiol*. B 2007, 147, 645-656
- McPhee S., Hodges LD, Wright PFA, Wynne PM, Kalafatis N, Harney DW. *Comp. Biochem. Physiol*. B 2007, 146, 346-356