Journal of Life Sciences 6 (2012) 411-417

Lipid Content and Fatty Acid Composition of the Clam *Rangia cuneata* (G.B. Sowerby, 1831) in Upper Barataria Estuarine, Louisiana

Wai Hing Wong[1, 2], Siu Gin Cheung[3] and Paul K.S. Shin[3]

1. Louisiana Universities Marine Consortium, 8124 Highway 56, Chauvin, LA 70343, USA

2. Department of Environmental and Occupational Health, University of Nevada, Las Vegas, 4505 Maryland Parkway Box 452040 Las Vegas NV 89154, USA

3. Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Ave, Hong Kong, China

Received: May 14, 2011 / Accepted: June 18, 2011 / Published: April 30, 2012.

Abstract: Rangia clam, *Rangia cuneata* (G.B. Sowerby, 1831), is an important economic species and traditional seafood for Louisiana local Indians and local fishery communities. Although it is popular along the Gulf of Mexico area, especially for American Indians and local fish communities, none of its nutritional value has been studied. We firstly reported the fatty acid composition of this clam. The contents of total lipid and neutral lipid in the soft tissue of the clam were 77.7 ± 0.7 and 43.7 ± 0.4 mg/g, respectively. The contents of saturated monoenoic and polyunsaturated fatty acids were 14.8, 8.0, and 8.3 mg/g, respectively, in total lipids, and 8.1, 4.5, and 4.8 mg/g, respectively, in neutral lipid. For the fatty acid composition, saturated fatty acids had the greatest content (47.3%), followed by polyunsaturated fatty acids (27.1%) and monoenoic acids (22.6%). This widely distributed bivalve in the Gulf of Mexico and Atlantic coast, often in high densities, has a more nutritious fatty acid composition than plant oils currently being tested as fish oil replacements in the aquaculture feed industry. It is suggested that this widely distributed bivalve may have potential as fish oil replacement in small scale aquaculture feed industry.

Key words: *Rangia cuneata*, clam, fatty acid composition, fatty acid comparison, replacement.

1. Introduction

Rangia cuneata (G.B. Sowerby, 1831), known as the Atlantic *Rangia*, *Rangia* clam, brackish water clam, or estuarine clam, is found along the Gulf of Mexico and Atlantic coast, as far north as New Jersey. It is an important component of estuarine ecosystems, occupying as much as 95% of the total benthic biomass [1, 2]. It cleans water by filtering suspended particles and provides a suitable substratum for attachment of epifauna [2]. Apart from its ecological values, the shell of *R. cuneata* has been used for roadbed materials and the manufacture of many industrial products. It was a popular food for houmas native Americans and early settlers of South Louisiana, and today is still consumed by local fishing communities [3]. In comparison with studies in other clam species in the United States, such as hard clam *Mercenaria mercenaria* and soft clam *Mya arenaria*, limited research has been conducted on the chemical composition and nutritional components of this ecologically and economically important clam species. Therefore, the nutritional value of this clam has been overlooked in the past. The objective of our study was to quantify fatty acid composition of the soft tissue of *R. cuneata* and further to compare its major fatty acid composition with other organisms, such as other bivalves, fishes, as well as some plants that are being

Corresponding author: Wai Hing Wong, Ph.D., associate research professor, research fields: aquatic biology and environmental health. E-mail: david.wong@unlv.edu.

tested as fish oil replacements in aquaculture industry.

2. Materials and Methods

2.1 Clam Collection and Sample Preparation

R. cuneata with shell lengths between 20 and 35 mm was selected from 10 locations in the upper Barataria estuary in Southeastern Louisiana in late May 2004. In each location, four to five individual clams were collected, cleaned and stored in a cooler. After being transported to the laboratory, the clams were frozen at -20 °C until further analysis. Prior to lipid extraction, the soft tissues were removed and rinsed with distilled water. The tissue of the four to five clams collected from each location was then freeze-dried for 72 hours (dura dry freeze-dryer, -75 °C). The dried tissue was passed through a sieve of MF-Sieb 0.5 mm (GmbH & Co. KG), and mixed by a homogenizer (model IKA®—WERKE).

2.2 Lipid Extraction and Quantification

For lipid extraction, about 250 mg of dry clam tissues from each location were used according to a modified method of Bligh and Dyer [4]. Lipid was extracted overnight in a 5 mL chloroform-methanol solvent mixture (2:1 v/v) in a pre-weighed centrifuge tube. The mixed crude extract was then washed with 0.04% $CaCl_2$ solution and centrifuged. The upper aqueous layer was removed and the lower layer was dried with a stream of nitrogen. Hexane (10 mL) was then added and the resulting solution was divided into two aliquots. The first aliquot was for determination of total lipid, which was dried with a stream of nitrogen and further dried overnight in a vacuum desiccator. Total lipids were determined gravimetrically. Cold acetone (3 mL) was added to the second aliquot to extract the neutral lipids. The weight of the neutral lipid fraction was obtained by transferring the acetone layer into a pre-weighed centrifuge tube, drying the tube and solution in a stream of nitrogen and then overnight in a vacuum desiccator, and subtracting the tube weight from the weight of the tube and residue.

2.3 Fatty Acid Analysis and Quantification

Fatty acid methyl esters (FAMEs) of total and neutral lipids were also determined following a modified method of Bligh and Dyer [4]. This modified method has been well established in our laboratory. Generally, 6% of H_2SO_4 in methanol were added to the lipid extract, and the solution was incubated in the oven at 80 °C for two hours. After cooling, 1 mL distilled water and 2 mL petroleum ether were added to the tube and mixed using a vortex. The upper organic layer was transferred to a vial and dried using a nitrogen stream with a very slow flow rate. FAMEs were analyzed using an Agilent 6890 series GC-FID, including an autosampler and SPB-PUFA capillary column (30 m, 0.32 mm internal diameter, 0.20 μm film thickness). Authentic methylated fatty acid standards were purchased from sigma and supelco, and they are all certified products. C19:0 (methyl nonadecanoate) was used as an internal standard. Standard FAMEs (supelco: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1n9c, C18:1n9t, C18:2n6c, C18:2n6t, C18:3n6, C18:3n3, C20:0, C20:1n9, C20:2, C20:3n6, C20:3n3, C20:4n6, C20:5n3, C21:0, C22:0, C22:1n9, C22:2, C22:6n3, C23:0, C24:0, and C24:1n9.) solution (10-200 ppm) was also prepared and added with 15 ppm of internal standard. These standard fatty acids were all separated and easily identified and the peak of each standard FAME was also checked with GC-MS [5]. For each standard FAME, a calibration curve between the peak area of the specific FAME and the peak area of the internal standard was established to calculate the concentration of the FAME in the lipid extract. The operating conditions for the GC-FID were as follows: split injection mode was used with injector being held at 250 °C; the temperature sequence was 50 °C for 2 min, 50 °C to 210 °C at 4 °C/min, and 210 °C for 50 min. The detector was held at 260 °C and helium was used as the carrier gas at a flow rate of 1 mL/min. Sample of 1 μL was injected into the GC-FID for each

analysis.

2.4 Statistical Analysis and Comparison of Fatty Acids with Other Sources

The fatty acid content of total lipid and neutral lipid in clam tissue were compared using analysis of variance (ANOVA). If significant differences were found, the data were further compared using post-hoc multiple comparison tests (student newman Keuls: SNK) [6]. The significance level of the test was set at the criterion of $\alpha = 0.05$. A cluster analysis was used to compare the similarities in the fatty acid composition (w/w %) of *R. cuneata* to other bivalves, fish, and plants. The data sources for these organisms are: mud clam *Geloina coaxans* [7], hard clam *Mercenaria mercenaria*, soft clam *Mya arenaria*, surf clam *Spisula solidissima* [8], hydrothermal vent clam *Calyptogena pacifica* [9], green mussel *Perna viridis* [10], Pacific oyster *Crasosstrea gigas* [11], eastern oyster *Crassostrea virginica* [8], wild sea bass and cultured sea bass [12], Atlantic salmon, tilapia, carp, catfish [13], anchovy, mackerel [14], and soy, canola, rapeseed, corn, hemp, linseed, palm, and lupin [15]. A Bray-Curtis similarity index was constructed after a square root transformation of the data [16].

3. Results and Discussion

3.1 Lipid Content and Fatty Acid Composition in Clam Tissue

The amounts of total lipid and neutral lipid were 77.8 ± 5.9 (mean ± SE, n = 10) and 43.7 ± 3.2 (mean ± SE, n = 10) mg/g, respectively. Neutral lipid was about 56% of the total lipid. Fourteen fatty acids were identified in the soft tissue of the *R. cuneata* (Table 1). The contents of fatty acids were between 0.47 (C20:3n3) and 8.30 (C16:0) mg/g in total lipid and 0.15 (C20:3n3) to 4.44 (C16:0) mg/g in neutral lipids. Analysis of variance (ANOVA) indicated that there was a significant difference ($P < 0.05$) in the absolute content of each fatty acid for either total or neutral lipid. Results of the SNK test established that the content of C16:0 was the greatest, followed by C18:0, C16:1, C22:6n3 (DHA: docosahexaenoic acid), C18:1n9c, and C20:5n3 (EPA: eicosapentaenoic acid). Other fatty acids were in relatively lower concentrations (Table 1). A similar trend was found for fatty acids in neutral lipid (Table 1).

Table 1 Content of fatty acids (mg/g DW) in total lipid and neutral lipid of *R. Cuneata* (n = 10, mean ± SE).

Fatty acids	Total lipid	Neutral lipid
C14:0	1.2 ± 0.1 d, e	0.7 ± 0.1 e, f, g
C15:0	0.48 ± 0.0 e	0.3 ± 0.0 g
C16:0	8.3 ± 0.7 a	4.4 ± 0.4 a
C17:0	1.5 ± 0.1 d, e	0.8 ± 0.1 e, f
C18:0	3.3 ± 0.2 b	1.9 ± 0.1 b
Total saturated	14.8	8.1
C16:1	3.07 ± 0.31 b	1.7 ± 0.2 b, c
C18:1n9c	2.5 ± 0.2 b, c	1.3 ± 0.1 c, d
C18:1n7	1.9 ± 0.2 c, d	1.1 ± 0.1 d, e
C20:1n9	0.6 ± 0.04 e	0.3 ± 0.0 f, g
Total monoenoic	8.0	4.5
C18:2n6c	1.3 ± 0.1 d, e	0.7 ± 0.1 e, f, g
C20:3n3	0.5 ± 0.2 e	0.2 ± 0.0 g
C20:4n6	1.8 ± 0.1 c, d	1.1 ± 0.1 d, e
C20:5n3	2.1 ± 0.2 c, d	1.3 ± 0.1 c, d
C22:6n3	2.6 ± 0.2 b, c	1.6 ± 0.1 b, c, d
Total polyunsaturated	8.3	4.9

Same letter denotes no significant difference in the same column ($P > 0.05$) among fatty acids from the SNK test.

Total saturated fatty acids comprised the highest percentage (47.3%) of the total fatty acids (w/w), followed by polyunsaturated fatty acids (27.1%) and total monoenoic acids (25.5%) (Table 2). The most abundant saturated fatty acids were C16:0 (55.8%) and C18:0 (22.8%). The most common monoenoic acids were C16:1 (37.8%) and C18:1n9c (23.2%). DHA (31.2%) and EPA (24.8%) were the most common unsaturated fatty acids. The total percentage of DHA and EPA was 15.2% (Table 2) and the n-3/n-6 ratio was 1.7. The composition of each fatty acid (% w/w of the total fatty acids) in neutral lipid was very similar to that in total lipid (Table 2), with the greatest percentage for saturated fatty acids and the lowest percentage for monoenoic acids (Table 2).

The content of total lipid in the soft tissues of *R. cuneata* (7.8%) is much greater than that in other bivalves, which is usually 1-2% of whole body tissue [17]. The sum of EPA and DHA (4.7 mg/g) is within the reported range in other shellfish species (2.0 to 8.4 mg/g) [18], but the total percentage of EPA and DHA (15.2%) is close to the low values for other bivalves (16.0 to 27.8%) reported by R.G. Ackman [19]. The ratio of n-3/n-6 (1.7) is also just below the values of other bivalves (2.0-15.4) [20]. The percentage of polyunsaturated fatty acids in this clam (27.1%) is smaller than that of most bivalves (40% and 60% [18]). This is possibly related with the feeding habit of this clam, as detritus, part of its food items (LaSalle and de la Cruz 1985), contains some bacteria which are not rich in polyunsaturated fatty acids. However, the percentage of polyunsaturated fatty acids in *R. cuneata* is greater than that of beef (5%) and pork (10%) [18]. Therefore, this calm still can provide substantial amounts of healthy fatty acids.

3.2 Comparison of Fatty Acid Profile of R. cuneata with Other Sources

The fatty acid composition of *R. cuneata* was compared with other bivalves, as well as some fish and plant oils. The Bray-Curtis similarity index of fatty acid composition shows that there are two similar groups (Fig. 1), one is the aquatic organisms (1-17 in Fig. 1) and the other is the plants (18-25 in Fig. 1). Among the aquatic species, fatty acid composition of *R. cuneata* is the most similar to wild sea bass at the similarity level of 90%, and then mud clam at the similarity level of 85% (Fig. 1). The fatty acid composition of *R. cuneata* shows higher similarity with most of the bivalve species (> 81%) than oils from aquatic organisms, with an exception of low similarity (58%) with sea hydrothermal vent clam (Fig. 1). The proportion (%) of EPA or DHA of this clam is intermediate among the bivalves and different fish species while there is no EPA or DHA in each of the plants (Table 3).

R. cuneata has a more similar fatty acid profile with the selected aquatic organisms than with oils of plants, which are currently being tested as a replacement or partial replacement of fish oils [21]. *R. cuneata* has a more similar fatty acid profile with fish than with plants. The low similarity between hydrothermal vent clams and *R. cuneata*, as well as other aquatic species is because deep-sea clam primarily relies on chemoautrophic

Table 2 Fatty acid composition (% w/w of total fatty acids) in total lipid and neutral lipid of *R. Cuneata* (n = 10, mean ± SE).

Fatty acids	Total lipid	Neutral lipid
Total saturated	47.3 ± 0.8	46.6 ± 0.6
Total monoenoic	25.5 ± 0.6	25.5 ± 0.5
Total polyunsaturated	27.1 ± 1.1	27.9 ± 0.7
EPA + DHA	15.2 ± 0.6	16.7 ± 0.8
Total n-3 fatty acids	16.8 ± 1.0	17.6 ± 0.8
Total n-6 fatty acids	10.3 ± 0.6	10.3 ± 0.4
n-3/n-6 ratio	1.7 ± 0.1	1.8 ± 0.1

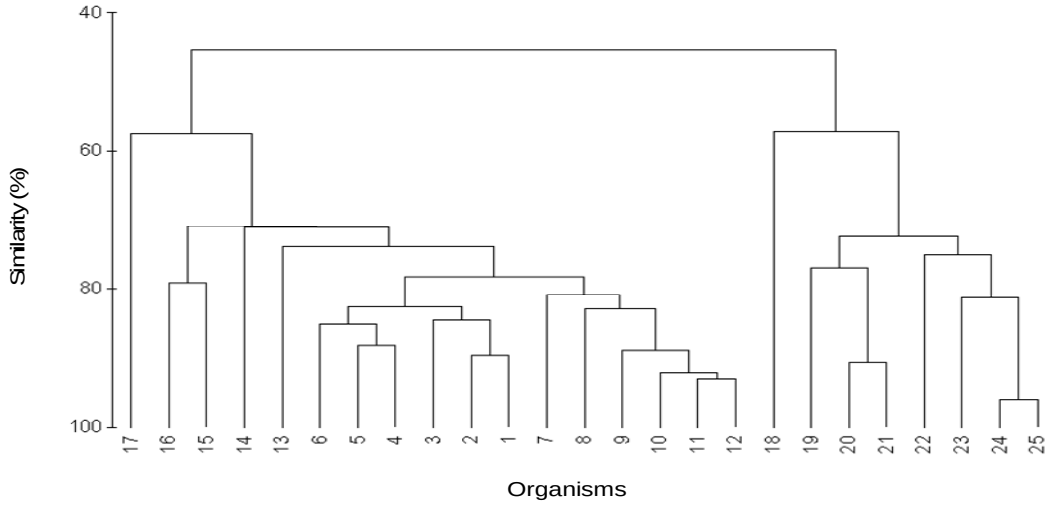

Fig. 1 Similarity (%) of major fatty acid composition (w/w %) among *R. cuneata* in the present experiment and other organisms. 1. *R. cuneata*; 2. sea bass (wild); 3. mud clam; 4. sea bass (cultured); 5. Atlantic salmon; 6. tilapia; 7. green mussel; 8. anchovy; 9. Eastern oyster; 10. hard clam; 11. soft clam; 12. surf clam; 13. Pacific oyster; 14. Carp; 15. mackerel; 16. catfish; 17. hydrothermal vent clam; 18. hemp; 19. rapeseed; 20. canola; 21. lupin; 22. palm; 23. linseed; 24. soy; 25. corn.

Table 3 EPA and DHA (% w/w of total fatty acids) of different sources (data are from the same resources as shown in Fig. 1).

	Organisms	EPA	DHA
Bivalve	*R. cuneata*	6.74	8.45
	Hydrothermal vent clam	0.31	0.14
	Mud clam	1.50	3.80
	Hard clam	4.50	15.10
	Soft clam	11.20	13.40
	Surf clam	11.40	11.50
	Pacific oyster	4.40	2.50
	Eastern oyster	9.90	15.40
	Green mussel	17.89	38.06
Fish	Anchovy	11.00	20.00
	Atlantic salmon	4.00	6.71
	Carp	8.67	0.77
	Catfish	0.19	2.22
	Mackerel	1.00	8.00
	Sea bass (cultured)	6.00	18.10
	Sea bass (wild)	10.60	19.50
	Tilapia	1.38	3.99
Terrestrial Plant	Canola	0.00	0.00
	Corn	0.00	0.00
	Hemp	0.00	0.00
	Linseed	0.00	0.00
	Lupin	0.00	0.00
	Palm	0.00	0.00
	Rapeseed	0.00	0.00
	Soy	0.00	0.00

symbionts in its gills for nutrition in its extreme deep-sea environment [9].

R. cuneata is abundant in coastal Louisiana. For example, in the oligohaline waters of southwestern Louisiana, it is estimated that the minimum standing crop of this clam is between 24 and 48 billion individuals [22]. Based on the tissue dry weight of this clam, estimated at 0.13 g per individual [23], the minimum production of lipid from this clam is at 243-485 tons just from southwestern Louisiana. The relatively greater content and percentage of polyunsaturated fatty acids (especially EPA and DHA) as compared to plant oil, as well as the relatively higher amount of total lipid, suggest that *R. cuneata* is a potential source of polyunsaturated fatty acids.

Shellfish have many benefits for human diets. They are good sources of high-quality protein, minerals (iron, zinc, and copper), and vitamin B_{12}. They are relatively low in saturated fat, and relatively high in polyunsaturated fatty acids [18]. The Louisiana Agriculture Experiment Station, the Louisiana Cooperative Extension Service, state and federal regulatory agencies, and commercial fishermen have been involved in efforts to develop *R. cuneata* as a marketable seafood that meets all the regulatory guidelines of microbial safety for molluscan shellfish established by the National Shellfish Sanitation Program (NSSP). It has been proposed that the *R. cuneata* fishery has a growth potential at least equal to the oyster fishery in Louisiana, and preliminary analysis has indicated that potential markets exist in Louisiana, on the east and west coasts of the United States, in Western Europe, and the far East [24]. However, the market for *R. cuneata* has been slow to develop due to its muddy flavor attributed to the compound geosmin [3].

Based on this clam's fatty acid analysis in the present study, it may also have the potential to be used as a source of oil in fish feed for small aquaculture operations. In aquaculture, fish meal and fish oil are expensive and they are from fishery stocks that are being depleted [25]. Fish oil is likely to become a limiting commodity sooner than fish meal in the aquaculture industry and many fish oil alternatives are currently being investigated [21]. The fatty acid composition of *R. cuneata* meets the fatty acid requirements of a fish diet better than most of the terrestrial plant oils currently being tested as fish oil replacements. Firstly, the ratio of n-3 fatty acids/n-6 fatty acids, an index of relative nutritional value of fatty acids for different sources of lipids [26], is higher in *R. cuneata* (1.7) as compared to plant-derived fish oil replacements, such as soy (0.13), canola (0.58), rapeseed (0.62), corn (0.01), hemp (0.34), palm (0.02), and lupin (0.68), and except linseed (4.20) [15]. Secondly, EPA and DHA, as the two important polyunsaturated fatty acids in fish nutrition, are rich in *R. cuneata* while neither of them is found in plants (Table 3). Finally, the fatty acid composition of *R. cuneata* is more similar to the fatty acid profile of fish than to plant oils (Fig. 1). Therefore, the fatty acid analysis indicates that *R. cuneata* should provide a superior source of lipid to fish aquaculture.

Overall, *R. cuneata* has higher content of total lipid than most of other bivalves. It has intermediate content of EPA and DHA within the range for other shellfish species and the total percentage of polyunsaturated fatty acid is relatively low comparing with other bivalves. *R. cuneata* has a more similar fatty acid profile with the selected aquatic organisms than with oils of plants. Comparing to plants, *R. cuneata* has a more similar fatty acid profile with fish. Given the fact is distributed with high abundance along the coastal Louisiana, apart from being used as a seafood for human beings, this clam may also have the potential to be partially used as fish feed by providing healthy fish oil.

Acknowledgments

The work described in this paper was supported by the US National Oceanic and Atmospheric Administration Grant (No. NA16OP2671) and

partially supported by a strategic grant from City University of Hong Kong (Project No. 7001284). Without support from Dr. Nancy Rabalais and Dr. Robert Gene Turner, this experiment could not be conducted. The constructive comments from Jodi Brandt are helpful in improving the quality of an early version of this manuscript.

References

[1] M.W. LaSalle, A.A. de la Cruz, Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico)—Common Rangia, Research and Development Branch of the U.S. Fish and Wildlife Service, 1985, pp. 1-18.

[2] W.H. Wong, N.N. Rabalais, R.E. Turner, Abundance and ecological significance of the clam *Rangia cuneata* (Sowerby, 1831) in the upper Barataria Estuary (Louisiana, USA), Hydrobiologia 651 (2010) 305-315.

[3] L.S. Andrews, R.M. Grodner, Muddy flavor and acceptability of fresh iced-stored and value added Louisiana Rangia clam, Journal of Food Quality 18 (1995) 445-453.

[4] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Canadian Journal of Biochemistry and Physiology 37 (1959) 911-917.

[5] Q.F. Gao, P.K.S. Shin, G.H. Lin, S.P. Chen, S.G. Cheung, Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system, Marine Ecology Progress Series 317 (2006) 273-283.

[6] J.H. Zar, Biostatistical Analysis, 3rd ed., Prentice Hall Inc., Upper Saddle River, New Jersey, USA, 1996, p. 662.

[7] Z. Bachok, P.L. Mfilinge, M. Tsuchiya, The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan, Journal of Experimental Marine Biology and Ecology 292 (2003) 187-197.

[8] J.C. Bonnet, V.D. Sidwell, E.G. Zook, Chemical and nutritive values of several fresh and canned finfish, crustaceans, and mollusks, Part II: Fatty acid composition, Marine Fisheries Review 36 (1974) 8-14.

[9] C.E. Allen, P.A. Tyler, C.L. Van Dover, Lipid composition of the hydrothermal vent clam *Calyptogena pacifica* (Mollusca: Bivalvia) as a trophic indicator, Journal of Marine Biolological Association 81 (2001) 817-821.

[10] K.Y. Chan, Q.F. Gao, K.M. Yip, W.H. Wong, P.K.S. Shin, S.G. Cheung, Lipid content and fatty acid composition in the green-lipped mussel *Perna viridis* (L.), Journal of Food Lipids 11 (2004) 123-130.

[11] M. Caers, S.D. Utting, P. Coutteau, P.F. Millican, P. Sorgeloos, Impact of the supplementation of a docosahexaenoic acid-rich emulsion on the reproductive output of oyster broodstock, *Crassostrea gigas*, Marine Biology 140 (2002) 1157-1166.

[12] C. Alasalvar, K.D.A. Taylor, E. Zubcovb, F. Shahidic, M. Alexisd, Differentiation of cultured and wild sea bass (Dicentrarchus labrax): Total lipid content, fatty acid and trace mineral composition, Food Chem. 79 (2002) 145-150.

[13] G.M. Pigott, B.W. Tucker, Science opens new horizons for marine lipids in human nutrition, Food Review International 3 (1987) 105-138.

[14] P. Karakoltsidis, A. Zotos, S.M. Constantinides, Composition of the commercially important Mediterranean finfish, crustaceans, and molluscs, Journal of Food Composition and Analysis 8 (1995) 258-273.

[15] D.A. Higgs, F.M. Dong, Lipids and fatty acids, in: R.R. Stickney (Ed.), Encyclopedia of Aquaculture, John Wiley & Sons, New York, 2000, pp. 476-496.

[16] K.R. Clarke, R.M. Warwick, Change in marine communities: An approach to statistical analysis and interpretation, Plymouth Marine Laboratory: Primer-E Ltd., 2001.

[17] I. King, M.T. Childs, C. Dorsett, J.G. Ostrander, E.R. Monsen, Shellfish: Proximate composition, minerals, fatty acid, and sterols, Journal of American Dietetic Association 90 (1990) 677-685.

[18] F.M. Dong, The Nutritional Value of Shellfish in Washington Sea Grant, Seattle, Washington, USA, 2001, pp. 1-8.

[19] R.G. Ackman, Composition and nutritional value of fish and shellfish lipids, in: A. Ruiter (Ed.), Fish and Fishery Products, Composition, Nutritive Properties and Stability, CAB International, Wallingford, Oxon, UK, 1995, pp 117-156.

[20] J. Surh, J.S. Ryu, H.J. Kwon, Seasonal variations of fatty acid compositions in various Korean shellfish, Journal of Agricultural and Food Chemistry 51 (2003) 1617-1622.

[21] R.W. Hardy, Conflict ahead: Can we reduce fish oil use?, Aquaculture Magazine 6 (2003) 44-48.

[22] H.D. Hoese, Abundance of the low salinity clam, *Rangia cuneata* in southwestern Louisiana, Proceedings of the National Shellfisheries Association 63 (1973) 99-106.

[23] W.H. Wong, N.N. Rabalais, Abundance and distribution of Rangia clams in three coastal lakes of southeastern Louisiana, Journal of Shellfish Research 24 (2005) 684.

[24] R.M. Grodner, L.S. Andrews, Advances in utilization of Rangia clam resources in Louisiana, Louisiana State University Agriculture Center Bulletin 843 (1992) 3-37.

[25] K.C. Jaehnig, Canola oil may offer multiple benefits to fish farmers, Aquaculture Magazine 30 (3) (2004) 11-12.

[26] G.M. Pigott, B.W. Tucker, Seafood: Effects of Technology on Nutrition. New York, USA, Marcel Dekker, 1990, p. 384.