Malays. Appl. Biol. (2014) 43(1): 31–39

FATTY ACID PROFILING OF BENTHIC HARPACTICOID (*Pararobertsonia* sp.) EXPOSED TO ENVIRONMENTAL STRESSES

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ABSTRACT

Effect of various environmental stresses on the fatty acid (FA) profile of benthic harpacticoid copepod (*Pararobertsonia* sp.) was checked *In vitro*. Samples were exposed to different pH (5, 7 and 9) and salinity (15, 20, 25, 30 and 35 ppt) at constant temperature 25ºC for 30 days. After the treatment, different fatty acid levels were determined using Gas Chromatography and Mass Spectrometry (GC-MS). Results clearly indicated the positive influence of the combined effect of environmental parameters on the fatty acid content in experimental samples. The detected FAs were ranging from C_{5-24}. Palmetic and oleic acids were in higher percentage in all the experiments. Results clearly indicated that pH7:25ppt & 35ppt at 25ºC ambient water temperature would help in producing copepods (*Pararobertsonia* sp.) that expresses rich fatty acid profile with high EPA/DHA ratio.

Key words: Fatty acid, harpacticoid copepod, *Pararobertsonia* sp., environmental stress

INTRODUCTION

Copepods are the key functional players as primary consumers for many organism including marine fish larvae. Due to its desirable body sizes, high lipid and fatty acid level compared to commercial/conventional larval feeds (Artemia and Rotifers), they are preferentially used by the aquaculture industries as a starter feed for the early larval stages of fishes. Many studies have reported the improved survival, better growth and pigmentation rate in developing fish larvae when they were fed with early stage of copepods (nauplii and copepodites) (Støttrup and Norsker, 1997; Copeman *et al*., 2002; Cutts, 2003; Vizcaíno-Ochoa *et al*., 2010; Ajiboye *et al*., 2011). This is generally due to the availability of high level of essential fatty acids such as Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA) and arachidomic acid (ARA) in copepods which are proven to be instrumental for better growth and survival of fish larvae (Tocher *et al*., 1989). Studies have shown that most marine fish larvae feeds on copepod eggs and nauplii stages during the first few weeks after the onset of exogenous feeding (Nanton and Castell, 1998) which helps the researches to use copepods as biomarkers of trophic transfer (Parrish *et al*., 2000; Dalsgaard *et al*., 2003; Zaleha *et al*., 2014).

It is well documented that the marine harpacticoids in tropical water are frequently experiencing sudden fluctuation in the salinity and ambient temperature round the year (Kassim Zaleha and Ibrahim Busra, 2012) Studies have shown that lowering the ambient temperature tends to increase the production of C_{20-22} polyunsaturated fatty acid production in planktonic crustacean (Nanton and Castell, 1998). However, the study to address the combined effect of other environmental parameters such as pH and salinity together with the temperature on the fatty acid content in benthic harpacticoid is still scanty (Zaleha and Farahiyah-Ilyana, 2010). Hence, the present study was aimed to investigate the combined effects of different pH and salinity condition [at static temperature (25ºC)] on the fatty acid content of benthic harpacticoid (*Pararobertsonia* sp.) cultured *in vitro*.

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MATERIALS AND METHODS

Sample collection and acclimation

Benthic harpacticoid (*Pararobertsonia* sp.) were collected from the stock culture (maintained at Live Feed Laboratory University Malaysia Terengganu) and acclimated in artificial sea water (35ppt), under continuous light for a month period. Samples were fed with ~2g of grated vegetable paste. Precautions were taken to avoid cross contamination from environmental sources.

Experimental setup

A total of 200 healthy adult individuals of *Pararobertsonia* sp. were transferred from stock culture to serially arranged each glass beakers (250ml). Water quality parameters were maintained as at pH5 (Salinity 15, 25, 35ppt), pH7 (Salinity 15, 25, 35ppt) and pH9 (Salinity 15, 25, 35ppt) in replicates. pH and salinity were measured using bench top pH probe and refractometer respectively. Water temperature was maintained at 25ºC in 24h light condition. Percentage mortality of experimental copepods was recorded in every 3 days (mortality ranged between ~10 and 60%). After 30 days of experimental period, the samples were freeze dried for fatty acid analysis.

Fatty acid analysis

Fatty acids (FAs) were extracted from freeze dried copepod samples using Püttman *et al*. (1993) for the qualitative and quantitative examination. The extracted FAs were transesterified in to fatty acid methyl esters (FAME) using only strong acid at 80-85ºC for about an hour. After this treatment, purified water and hexane were added and the upper organic layer was transferred to a vial (Ichihara and Fukubayashi, 2010). This step was performed several times to achieve complete extraction of FAME. Samples were then dried and dissolved again in 20 ul hexane to get 50 times concentration and to remove all solvent peaks (toluen). The concentrated samples were then injected into Gas chromatography–mass spectrometry (GC-MS) to read the spectra using caprylic acid ($CH_3(CH_2)_6COOH$) as an internal standard. Percentage of fatty acids detected in each treatment was expressed in Mean±SD. Analysis of Variance (ANOVA) test was performed to check the difference in FAs percentage between treatments.

RESULTS AND DISCUSSION

In the wild, copepods are the major portion of zooplankton biomass and thus playing as a natural food resource for juvenile fishes (Lahnsteiner *et al*., 2009). Many attempts were made to determine the lipid classes (LCs) and fatty acid (FAs) profile of wild and hatchery reared copepods (Fraser *et al*., 1989; Olsen *et al*., 1991; Vander Meeren *et al*., 1993; Norsker and Støttrup 1994; Evjemo *et al*., 2003; Drillet *et al*., 2006; Parrish *et al*., 2012). However they failed to address the effect of various water quality parameters on the expression level of LCs and FAs. A recent review on the reproductive biology of hatchery reared copepod has clearly proven that the water temperature and salinity could be the limiting factors for the egg hatching success and survival rate (Zaleha and Farahiyah, 2010). Considering this fact, in the present study, we hypothesized that the copepod cultured under different environmental conditions will be different in their fatty acid profile.

Wide ranges of essential fatty acids (EFAs) and non-essential fatty acids (nEFAs) were detected in different treatments with the carbon numbers ranging from $C_{5\text{-}24}$. Significant difference in fatty acid composition was observed between treatments ($p < 0.05$). Our results showed the quantitative and qualitative expression of fatty acids increased in pH7:25ppt, pH:7:35ppt, pH9:25ppt and pH9:35ppt treatments. EFAs such as Arachidonic Acid, alpha, gamma-Linolenic Acid and Ricinoleic Acid contributed in lower percentage (~0.13-0.81%) while Gondoic Acid was comparatively in higher level (~1.55-3.29%) in all treatments. Generally EPA and DHA were not detected in pH5, pH7: Sal 15 and pH9: Sal 35 treatments while the percentage of EPA and DHA was lower in all other treatments (~0.2-1.7%). EPA/DHA ratio was higher in pH7: Sal 35 treatment (>2) followed by pH7: Sal 25 (0.5) and lower in other treatments (<0.5). Studies have shown that more EPA/DHA level in the feed would lead to autoxidation in the juvenile fishes that eventually increase their survival rate (Gunstone and Norris, 1983). The presence of eicosenoic acid was noted in all treatment with significantly varying concentration ($p < 0.05$) (Table 1).

In case of nEFAs, palmetic, Oleic and Stearic acids were contributing ~0.2-28%, 0.4-34% and ~0.14-21% respectively. Tetradecanoic acid, 9-Hexadeceonic and Heptadecanoic acids were quantitated in all treatments with significantly varying concentration ($P<0.05$) while other fatty acids were scattered in different treatments with varying concentrations. Low percentage composition of Stearic, Mead, Margaric, Heneicosylic, Behenic , Tricosylic, Lignoceric and Nonadecyclic acids were noted only in pH7:25, pH35 and pH 9 treatments and completely absent in pH 5 and pH7:15 treatments (Table 2). We also detected some long chain acid (eg., C_{69}) and toxic acids such as Pelargonic acid (Table 3). This might probably be due to the interaction of strong acid used in the extraction method. However, the method

Table 1. Mean percentage composition of essential fatty acids detected in benthic harpacticoid exposed to different treatments. (Data represented in Mean±SD)

Group	Common Names	Structure Name/ Molecules Structure	Samples							
			pH 5: Sal 25	pH 5: Sal 35	pH 7: Sal 15	pH7: Sal 25	pH7: Sal 35	pH 9: Sal 15	pH 9: Sal 25	pH 9: Sal 35
Essential Fatty Acids	Arachidonic Acid	5,8,11,14-Eicosatetraenoic acid ($C_{20}H_{32}O_2$)	-	-	-	0.48±0.17^a	0.35±0.18^b	-	0.47±0.14^a	-
	alpha-Linolenic Acid	9,12,15-Octadecatrienoic acid ($C_{18}H_{30}O_2$)	-	-	-	-	0.13±0.01^a	-	0.24±0.11^b	-
	gamma-Linolenic Acid	6,9,12-Octadecatrienoic acid ($C_{18}H_{30}O_2$)	-	-	-	-	-	-	-	0.23±0.11
	Ricinoleic Acid	11-13 Eicosenoic acid ($C_{18}H_{34}O_3$)	-	-	-	0.81±0.32^a	0.31±0.06^b	-	-	-
	Gondoic Acid	11- Eicosenoic acid ($C_{20}H_{38}O_2$)	1.55±0.21^a	1.83±0.06^a	3.06±1.34^b	3.13±1.56^b	2.87±1.52^b	2.33±1.45^c	2.15±1.42^c	3.29±1.32^b
	EPA, Timnodonic Acid	5,8,11,14,17-Eicosapentaenoic acid ($C_{20}H_{30}O_2$)	-	-	-	0.20±0.13^a	0.39±0.11^a	0.42±0.11^b	0.67±0.32^c	-
	DHA, Cervonic acid	4,7,10,13,16,19-Docosahexaenoic acid (C_{22}:6 (n-3))	-	-	-	0.40±0.11^a	0.19±0.05^b	1.70±0.84^c	0.89±0.23^d	-
		Eicosenoic acid ($C_{20}H_{38}O_2$)	0.38±0.02^a	0.78±0.08^b	0.40±0.17^a	0.79±0.21^b	0.86±0.36^b	0.79±0.21^b	0.85±0.19^b	1.11±0.95^c
		8, 11-Eicosadienoic acid (C_{20}:2 (n-6))	-	-	-	0.61±0.22	-	-		-
		10,13-Eicosadienoic acid (C_{20}:2 (n-6))	-	-	-	-	-	-	0.74±0.27	-

Note : Different letters within a row represent a significant difference among groups.

Table 2. Mean percentage composition of Non- essential fatty acids detected in benthic harpacticoid exposed to different treatments. (Data represented in Mean±SD)

Group	Common Names	Structure Name	Samples							
			pH 5: Sal 25	pH 5: Sal 35	pH 7: Sal 15	pH7: Sal 25	pH7: Sal 35	pH 9: Sal 15	pH 9: Sal 25	pH 9: Sal 35
Non-essential Fatty Acids	Pimelic Acid	Heptanedioic acid, 4-methyl-	-	-	-	-	-	-	-	0.060.01
	Lauric Acid	Dodecanoic acid	0.42±0.14^{a}	0.15±0.06^{b}	-	0.13±0.05^{b}	0.16±0.05^{b}	0.19±0.05^{b}	0.53±0.17^{c}	0.24±0.12^{c}
		Dodecanoic acid, 4-methyl-	0.59±0.23^{a}	-	-	-	-	-	0.15±0.04^{b}	0.21±0.06^{c}
	Azelaic Acid	Nonanedioic acid	0.07±0.23^{a}	-	-	0.12±0.07^{a}	-	-	0.42±0.21^{b}	-
	Capric Acid	Decanoic acid	-	-	0.16±0.03	-	-	-	-	-
	Sebacic Acid	Decanedioic acid	0.38±0.12^{a}	-	1.00±0.22^{b}	-	0.08±0.03^{c}	0.41±0.14^{a}	0.45±0.11^{a}	0.14±0.08^{c}
	Oleic Acid	9-Octadecenoic acid, 12-(acetyloxy)-	-	-	-	-	0.05±0.01	-	-	-
	Myristic Acid	Tetradecanoic acid	1.92±0.56^{a}	1.64±0.44^{a}	1.72±0.45^{a}	0.84±0.27^{b}	1.01±0.37^{b}	0.82±0.43^{b}	1.18±0.66^{b}	1.46±0.26^{a}
		Tetradecanoic acid, 12-methyl-	0.74±0.05^{a}	0.60±0.28^{a}	-	0.41±0.15^{b}	0.61±0.73^{a}	0.18±0.02^{c}	0.69±0.02^{a}	0.66±0.33^{a}
	Capric Acid	Decanoic acid, 3-methyl-	-	-	-	0.11±0.03^{a}	0.13±0.02^{a}	-	-	-
		Pentadecanoic acid	2.49±0.11^{a}	0.87±0.21^{b}	-	0.70±0.42^{b}	0.97±0.28^{b}	0.32±0.18^{c}	1.28±0.94^{d}	1.21±0.49^{d}
		Pentadecanoic acid, 14-methyl-	1.86±0.27^{a}	1.37±0.57^{b}	-	0.82±0.31^{c}	0.73±0.42^{c}	-	-	1.07±0.38^{d}
		9-Dodecenoic acid	-	-	-	0.49±0.16^{a}	0.65±0.11^{b}	-	0.66±0.23^{b}	-
		Hexadecadienoic acid	-	-	-	-	0.43±0.13	-	-	-
		7-Hexadecenoic acid	0.86±0.15^{a}	-	-	-	1.59±0.59^{b}	0.28±0.11^{c}	-	6.01±2.11^{d}
		9-Hexadeceonic acid	1.76±0.56^{a}	4.41±2.11^{b}	0.40±0.16^{c}	3.04±1.45^{c}	2.47±0.14^{c}	1.24±0.09^{a}	4.90±2.1^{b}	1.19±0.32^{a}
		11-Hexadecenoic acid, 15-methyl-	-	-	-	-	-	-	0.34±0.16	-
	Palmitic Acid	Hexadecanoic acid	25.25±5.23^{a}	28.21±11.22^{a}	26.78±7.07^{a}	17.60±4.07^{b}	14.69±4.92^{b}	20.82±6.92^{b}	24.26±13.4^{a}	19.07±5.96^{b}
		Hexadecanoic acid, 15-methyl-	-	-	-	-	-	-	-	2.83±1.22
		Hexadecanoic acid, 14-methyl-	1.84±0.68^{a}	3.24±1.23^{b}	-	1.95±0.37^{a}	1.02±0.49^{c}	0.41±0.11^{d}	2.89±1.22^{b}	1.21±0.91^{c}
		7-10 Hexadecanoic acid	-	-	-	0.28±0.08	-	-	-	-
	Crotonic Acid	2-Butenoic acid, 4-hydroxy-	-	-	-	-	0.37±0.18	-	-	-

	Margaric Acid	Heptadecanoic acid	1.86±0.36[a]	3.44±1.27[b]	0.26±0.01[c]	1.87±0.32[a]	1.77±0.79[a]	1.64±0.78[a]	2.66±0.94[d]	2.08±1.26[d]
		Heptadecanoic acid, 16-methyl-	0.35±0.04[a]	-	-	-	-	-	-	0.47±0.06[a]
	Linodelaic Acid	9,12-Octadecadienoic acid	2.24±1.11[a]	-	-	0.36±0.15[b]	0.48±0.14[b]	3.15±1.84[c]	9.65±3.11[d]	2.58±0.99[a]
		8,11-Octadecadienoic acid	-	-	-	5.92±1.00[a]	5.18±3.78[a]	-	-	-
		9,15-Octadecadienoic acid	-	2.19±1.12[a]	-	-	-	-	-	0.21±0.11[b]
		10,13-Octadecadienoic acid	-	1.68±.0.53[a]	-	-	-	-	12.19±4.21[b]	2.27±1.1[a]
		7,10-Octadecadienoic acid	-	-	-	-	0.30±0.11	-	-	-
	Oleic Acid	9-Octadecenoic acid (Z)-	24.68±6.29[a]	24.45±9.28[a]	34.69±8.72[b]	28.28±5.98[b]	27.88±9.69[a]	30.20±12.74[b]	22.71±9.46[a]	27.28±12.59[b]
		8-Octadecenoic acid (Z)-	18.12±4.92[a]	-	-	-	16.77±6.76[a]	-	-	-
		13-Octadecenoic acid (Z)-	-	-	-	-	-	19.76±9.12	-	-
		11-Octadecenoic acid	12.05±3.33[a]	10.59±2.95[a]	19.56±4.67[b]	13.84±4.43[a]	13.44±7.26[a]	5.86±2.91[c]	4.18±1.72[c]	11.54±4.84[a]
		15-Octadecenoic acid	0.14±0.01	-	-	-	-	-	-	-
	Stearic Acid	Octadecanoic acid	14.58±5.21[a]	21.86±7.87[b]	13.98±2.98[a]	15.49±5.32[a]	10.62±4.91[a]	20.33±8.16[b]	19.41±3.34[b]	13.47±3.95[a]
		Octadecanoic acid, 11-methyl-	-	-	-	0.18±0.01[a]	0.19±0.04[a]	-	-	0.14±0.01[b]
		Octadecanoic acid, 10-methyl-	-	-	-	-	0.30±0.17	-	-	-
		Octadecanoic acid, 17-methyl-	-	-	-	-	-	-	0.34±0.01	-
	Mead Acid	7,10,13-Eicosatrienoic acid		-	-	0.35±0.11[a]	0.19±0.02[b]	-	0.34±0.18[a]	-
		5,8,11-Eicosatrienoic acid	-	-	-	0.52±0.22[a]	-	-	0.39±0.13[a]	-
	Margaric Acid	Heptadecanoic acid, 4,8,12,16-tetramethyl-	-	-	-	-	-	-	0.08±0.02	-
	Heneicosylic acid	Heneicosanoic acid	-	-	-	-	0.18±0.01[a]	-	-	0.19±0.01[a]
	Behenic Acid	Docosanoic acid	-	-	-	0.37±0.15[a]	0.40±0.17[a]	-	0.49±0.12[b]	0.64±0.21[c]
	Tricosylic Acid	Tricosanoic acid	-	-	-	0.43±0.16[a]	0.59±0.27[b]	-	0.66±0.23[b]	0.99±0.25[c]
	Lignoceric Acid	Tetracosanoic acid	-	-	-	1.08±0.42[a]	0.87±0.83[b]	0.71±0.25[b]	1.57±0.96[c]	1.35±0.63[c]
	Nonadecyclic Acid	10-Nonadecenoic acid	-	-	-	-	0.08±0.01	-	-	-
		Nonadecanoic acid	-	-	-	0.18±0.05[a]	0.19±0.07[a]	0.26±0.11[b]	0.34±0.14[c]	0.31±0.14[c]

Note : Different letters within a row represent a significant difference among groups.

Table 3. Mean percentage composition of some long chain acids detected due to strong acid interaction (Data represented in Mean±SD)

Group	Common Names	Structure Name	Samples							
			pH 5: Sal 25	pH 5: Sal 35	pH 7: Sal 15	pH7: Sal 25	pH7: Sal 35	pH 9: Sal 15	pH 9: Sal 25	pH 9: Sal 35
Others	Pelargonic Acid	Nonanoic acid	0.23±0.11[a]	-	-	-	-	-	-	1.09±0.08[b]
		Nonanoic acid, 9-oxo-	-	-	-	-	-	-	0.09±0.03	-
	Undecyclic Acid	Undecanoic acid, 10-methyl-	-	-	0.27±0.01[a]	-	0.26±0.1[a]	-	-	0.45±0.16[b]
	Dicarboxylic Acid	Undecanedioic acid	-	-	-	-	-	-		0.26±0.11
	Tridecyclic Acid	Tridecanoic acid	-	-	-	-	-	-	1.05±0.29	-
		Tridecanoic, acid 12 methyl	-	-	-	-	-	-	1.18±0.83	-
		Cyclopropaneoctanoic acid, 2-hexyl	0.63±0.31[a]	-	-	0.43±0.09[b]	0.36±0.13[c]	-	-	0.20±0.09[d]
		Cyclopropaneoctanoic acid, 2-octyl	-	-	-	0.15±0.02[a]	-	-	0.77±0.34[b]	0.37±0.03[c]
		Nonahexacontanoic acid	-	-	-	-	0.06±0.01[a]	0.07±0.01[a]	-	-

Note : Different letters within a row represent a significant difference among groups.

adopted in this research gave quality peaks in chromatograms at the same retention time for each fatty acid detected, thus the method used in this study is highly reproducible.

Fatty Acids Composition

The FA composition of *Pararobertsonia* sp. was calculated from Table 1 & 2. In all experimental setup, the expression of C_{18} was higher (> 60%) followed by C_{16} (~20%). C_{15} and C_{17} were recorded in lower percentage (< 5%) (Fig. 1a). The higher expression level of C_{20} was noted in p7:25ppt, p7:35ppt and p9:25ppt experimental setups (Fig. 1b). $C_{21\text{-}24}$ were not detected in pH5 and pH7:15ppt treatments which might probably be due to the lower physiological and biochemical response by the cultured copepod under high stress conditions (Zaleha and Farahiyah-Ilyana 2010). Comparison with other studies on FA composition in wild and cultured *Calanus* sp. showed that the level of FAs in benthic harpacticoid (*Pararobertsonia* sp) is comparatively lower than the *Calanus* sp. (Parrish *et al*., 2012).

Overall, the observation showed that the expression of fatty acid is higher at the optimum culture conditions (pH7: 25 & 35ppt and pH9: 25ppt) compared to other treatments. This might probably due to the minimal stress undergone by the cultured copepods at these optimum conditions that eventually reflected in rich FA profile. Similar observation was reported in earlier reports (Zaleha and Farahiyah, 2010; Kassim Zaleha and Ibrahim Busra, 2012) where the copepod under gone minimal stress during the culture period and produced good survival and egg hatching success.

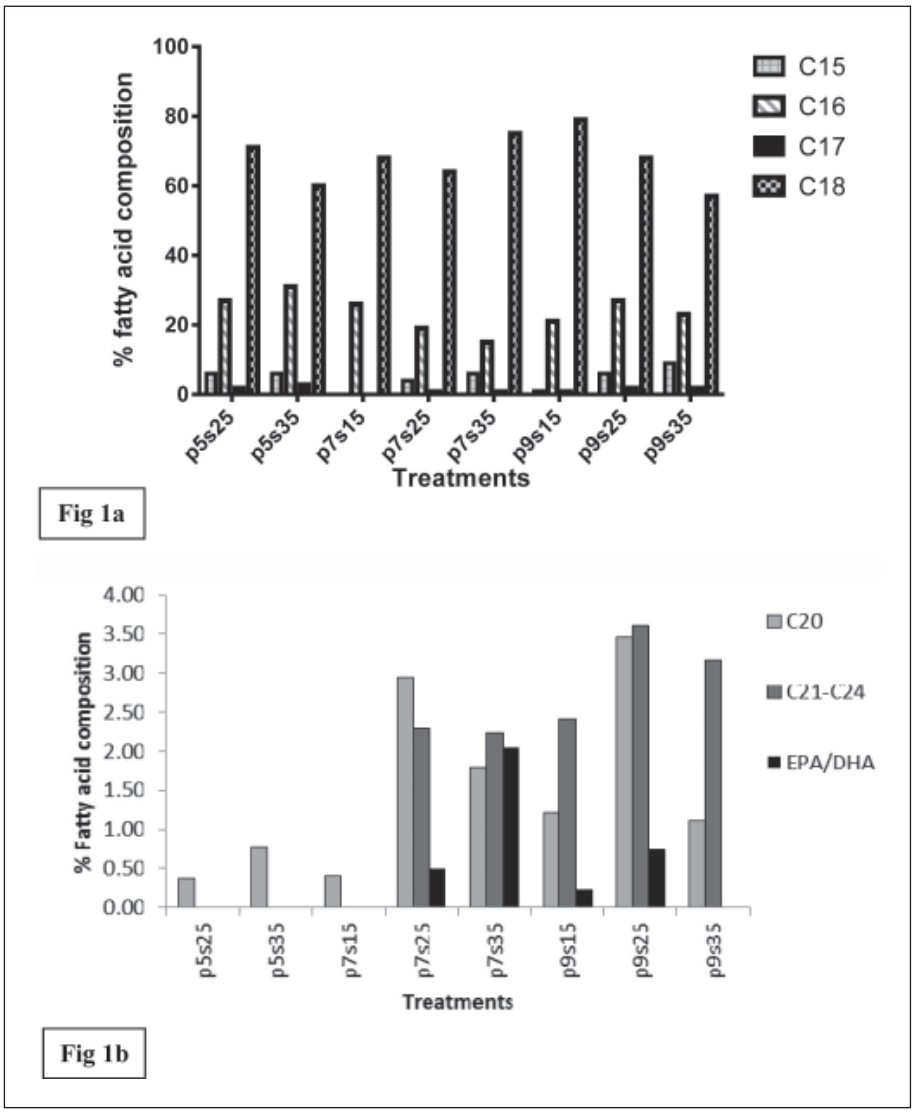

Fig. 1. Comparison of percentage fatty acid composition in benthic harpacticoid (*Pararobertsonia* sp.) cultured under different environmental conditions.
Note: P = pH; S = Salinity; C = Carbon numbers.

CONCLUSION

We were able to detect/quantify series of fatty acids ranging from EFAs EPA, DHA and many other nEFAs in various treatment conditions. The optimal culture condition for the production of hatchery reared copepods with sound fatty acid profile would be pH7 (or) pH25 with 35ppt at 25ºC ambient water temperature. However, the reproductive biology, generation time and survival rate should be taken in to consideration for the up scaling of hatchery reared copepods in treatment conditions.

ACKNOWLEDGEMENT

We extend our sincere thanks to Ministry of Science, Technology and Innovation (MOSTI) for funding this project.

REFERENCES

Ajiboye, O.O., Yakubu, A.F., Adams, T.E., Olaji, E.D. & Nwogu, N.A. 2011. A review of the use of copepods in marine fish larviculture. Reviews in Fish Biology and Fisheries, **21**(2), 225-246. doi: 10.1007/s11160-010-9169-3

Copeman, L.A., Parrish, C.C., Brown, J.A. & Harel, M. 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture, **210**(1–4), 285-304. doi: 10.1016/s0044-8486(01)00849-3

Cutts, C.J. 2003. Culture of harpacticoid copepods: Potential as live feed for rearing marine fish Advances in Marine Biology **44**: 295-316.

Dalsgaard, J., St John, M., Kattner, G., Müller-Navarra, D. & Hagen, W. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology, **46**: 225-340

Drillet, G., Jørgensen, N.O.G., Sørensen, T.F., Ramløv, H. & Hansen, B.W. 2006. Biochemical and technical observations supporting the use of copepods as live feed organisms in marine larviculture. Aquaculture Research, **37**(8), 756-772. doi: 10.1111/j.1365-2109.2006.01489.x

Evjemo, J.O., Reitan, K.I. & Olsen, Y. 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. Aquaculture, **227**(1–4), 191-210. doi: 10.1016/s0044-8486(03)00503-9

Fraser, A.J., Sargent, J.R., Gamble, J.C. & Seaton, D.D. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Marine Chemistry, **27**(1–2), 1-18. doi: 10.1016/0304-4203(89)90024-8

Gunstone, F.D. & Norris, F.P. 1983. Lipids in Foods. Pergamon Press, New York.

Ichihara, K. & Fukubayashi, Y. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. Journal of Lipid Research. **51**(3): 635-40.

Kassim Zaleha & Ibrahim Busra. 2012. Culture of Harpacticoid Copepods: Understanding the Reproduction and Effect of Environmental Factors, Aquaculture, Dr. Zainal Muchlisin (Ed.), ISBN: 978-953-307-974-5, InTech, Available from: http://www.intechopen.com/books/aquaculture/copepods-in-aquaculture

Lahnsteiner, F., Kletzl, M. & Weismann, T. 2009. The risk of parasite transfer to juvenile fishes by live copepod food with the example *Triaenophorus crassus* and *Triaenophorus nodulosus*. Aquaculture, **295**(1–2): 120-125. doi: 10.1016/j.aquaculture.2009.06.038

Nanton, D.A. & Castell, J.D. 1998. The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe sp*., for use as a live food for marine fish larvae. Aquaculture, **163**(3–4), 251-261. doi: 10.1016/s0044-8486(98)00236-1

Norsker, N.-H. & Støttrup, J.G. 1994. The importance of dietary HUFAs for fecundity and HUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture, **125**(1–2), 155-166. doi: 10.1016/0044-8486(94)90292-5

Olsen, R.E., Henderson, R.J. & Pedersen, T. 1991. The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. Journal of Experimental Marine Biology and Ecology, **148**(1), 59-76. doi: 10.1016/0022-0981(91)90147-o

Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., Pulchan, K. & Ramos, C. 2000. Lipid and Phenolic Biomarkers in Marine Ecosystems: Analysis and Applications. In P.J. Wangersky (Ed.), Marine Chemistry **5**: 193-223

Parrish, C.C., French, V.M. & Whiticar, M.J. 2012. Lipid class and fatty acid composition of copepods (*Calanus finmarchicus, C. glacialis, Pseudocalanus sp., Tisbe furcata and Nitokra lacustris*) fed various combinations of autotrophic and heterotrophic protists. Journal of Plankton Research, **34**(5), 356-375. doi: 10.1093/plankt/fbs003

Püttmann, M., Krug, H., von Ochsenstein, E. & Kattermann, R. 1993. Fast HPLC Determination of Serum Free Fatty Acids in the Picomole Range. Clinical chemistry **39**: 825-32.

Støttrup, J.G. & Norsker, N.H. 1997. Production and use of copepods in marine fish larviculture. Aquaculture, **155**(1–4), 231-247. doi: 10.1016/s0044-8486(97)00120-8

Tocher, D.R., Carr, J. & Sargent, J.R. 1989. Polyunsaturated fatty acid metabolism in fish cells: differential metabolism of (n-3) and (n-6) series acids by cultured cells orginating from a freshwater teleost fish and from a marine teleost fish. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, **94**(2), 367-374. doi: 10.1016/0305-0491(89)90357-x

van der Meeren, T., Wilhelmsen, S., Klungsosyr, J. & Kvenseth, P.G. 1993. Fatty Acid Composition of Unfed Cod Larvae *Gadus morhua* L. and Cod Larvae Feeding on Natural Plankton in Large Enclosures. Journal of the World Aquaculture Society, **24**(2), 167-185. doi: 10.1111/j.1749-7345.1993.tb00006.x

Vizcaíno-Ochoa, V., Lazo, J.P., Barón-Sevilla, B. & Drawbridge, M.A. 2010. The effect of dietary docosahexaenoic acid (DHA) on growth, survival and pigmentation of California halibut *Paralichthys californicus* larvae (Ayres, 1810). Aquaculture, **302**(3–4), 228-234. doi: 10.1016/j.aquaculture.2010.02.022

Zaleha, K. & Farahiyah-Ilyana, J. 2010. Culture and growth of a marine harpacticoid, *Pararobertsonia* sp. in different salinity and temperature. Sains Malaysiana **39**(1): 137-141.

Zaleha, K., Akbar John, B., Lim Keng, C., Nur Farahiyah, Z. & Nur Hidayah, A. 2014. Sustainable Technique for Selected Live Feed Culture. *In Sustainable Aquaculture Techniques.* Martha Hernandez-Vergara (Edi.), ISBN: 978-953-51-1224-2, InTech, doi: 10.5772/57212. Available from: http://www.intechopen.com/books/sustainable-aquaculture-techniques/sustainable-technique-for-selected-live-feed-culture