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Comparative fatty acid analysis of wild and cultured freshwater harpacticoid copepod – *Onychocamptus mohammed* (Blanchard and Richard, 1891)

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***Abstract*: The fatty acid profile of *Onychocamptus mohammed* (Blanchard and Richard 1891) from laboratory culture and the wild species from the habitat was ascertained using gas chromatography–mass spectrometry (GC-MS) and compared. The fatty acids detected in this experiment have the carbon number ranging between C_{14-25}. The wild sample showed the presence of 24 fatty acids in which the highest percentage was shown by nonadecanoic acid, methyl ester (2.25 ± 0.27%) and the lowest was shown by 15-tetracosenoic acid, methyl ester, (Z)- (0.04 ± 0.01 %); tetracosanoic acid, methyl ester (0.04 ± 0.02 %) and 5,8,11,14,17-eicosapentaenoic acid, methyl ester, (all-Z)- (0.04 ± 0.05 %). In cultured sample, 26 fatty acids were present and the highest percentage was shown by methyl hexadec-9-enoate (2.21 ± 0.03 %) and the least percentage was shown by heptadecanoic acid, methyl ester (0.05 ± 0.02 %) and heptadecanoic acid,16-methyl-, methyl ester (0.05 ± 0.01 %). The essential fatty acids such as docosahexaenoic acid (1.34 ± 0.19 %) and eicosapentaenoic acid (1.85 ± 0.01 %) were present in desired quantities in cultured sample and this ensures that the harpacticoid copepod, *O. mohammed* can serve as a live feed for developing shell fish and fin fish larvae.**

***Keywords*: fatty acid, live food, harpacticoid copepod.**

I. INTRODUCTION

In aquaculture, the nutritional aspect of the reared shellfish and finfish are of chief concern because it has an immense impact on growth, production and the survival rate of those cultured aquatic organisms. Larval rearing is one of the toughest periods in aquaculture and this is due to the incident of high mortality rate, but it can be overcome by providing them proper diet. The success of aquaculture depends on healthy cultured stock. Such disease free healthy stocks can be maintained by feeding them with live food (Das *et al*., 2012).

The developing fish larvae require food having comparatively high concentrations of the long - chain, polyunsaturated or essential fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachinoid acid (ARA) (Watanabe *et al*., 1982). The deficiency of DHA leads to impaired vision, malpigmentation and skeletal deformities and insufficiency of EPA and ARA results in stress vulnerability and affects the immune response (Tocher, 2003). The required ratio of DHA, EPA and ARA for the normal development of the fish is given as 10:5:1 by Sargent *et al.* (1997).

Harpacticoid copepods are the group with various important features favoring aquaculture and hence they carry a live feed tag. They measure around 80 µm to > 900 µm (first nauplius to adult) in length, so they can be ingested by smaller stages of fish and prawn larvae (Gee, 1989). They are a natural synthesizer of essential fatty acids like polyunsaturated fatty acid (PUFA) *viz*., EPA and DHA even when they are fed with nutritionally poor food (Arndt, 2013). They can endure wide range of salinity and temperatures (Hagiwara *et al.*, 1995; Zaleha & Busra, 2012). They can feed on diverse food sources

like small microalgae, ciliates, fungi, bacteria, vegetables, rice grains, and dead matter of plants and animals (Rajthilak *et al*., 2014). Moreover, they are less prone to infections (Michajlow, 1969). As per Hagiwara *et al*. (1995) the copepod species that are chosen as live feed should possess the following demographic characters like high reproducing capacity, larger brood size, longer reproducing duration, more female population, shorter generation time, shorter turnover time, faster growth and high survival rate. All these features are possessed by certain harpacticoid copepod (Uhlig, 1984; Saboor & Altaff, 2012).

In the present study, the identified harpacticoid species from Puzhal Lake, Chennai was cultured and its fatty acid profile was ascertained. Simultaneously, the wild harpacticoid sample from the habitat was also subjected to fatty acid analysis and the results were compared.

II. MATERIALS AND METHODS

Individual pair of harpacticoids were cultured in a rectangular shape glass tank packed with mud substratum containing filtered habitat water. They were provided with 12 L : 12 D photoperiod, fed with *C. striolatum* (5 x 10^6 cells / ml) and maintained at 25 ºC. This culture condition was derived by conducting preliminary experiments.

Adult harpacticoid copepods from the culture were collected on 200 µm mesh nylon cloth and introduced in to a beaker containing filtered, UV - treated habitat water. They were starved for 24 hrs for clearing the gut. Further, the copepods were suction-filtered on Whatman no. 1 filter paper and rinsed with distilled water. The filtered harpacticoid specimen was added to 10 ml and 5 ml of chloroform : methanol (2 : l v/v) respectively and were homogenized in a 20 ml glass culture tube using a polytron following Nanton (1997).

For extracting the total lipids, 2 ml of 7 % boron trifluoride and 0.5 ml of toluene per 1 mg of fatty acid were added. Further, it was boiled for 30 minutes at 100 °C in pressure - tested 15 ml teflon-lined screw cap culture tubes. The tubes were allowed to cool and 10 ml of distilled water was added to it. The top hexane layer was extracted twice with 2 to 3 ml of hexane and water layer were discarded. The hexane was dried with anhydrous sodium sulfate and filtered it through the Pasteur pipette containing the glass wool. The evaporation of hexane was carried out with nitrogen till it completely dries. Then, 30 µL of chloroform was added to 1 mg of sample and a streak of 1 cm was applied from the bottom measuring 2.5 cm of a precoated silica gel thin layer chromatography (TLC) plate. Also 15 µL of a reference methyl ester sample was added on the side of the plate, 2.5 cm from the bottom. The plate was kept in the mixture of hexane: diethyl ether: acetic acid (90 : 10 : l V/V/V) for 45 minutes that enabled the solvent to evaporate.

The section of the plate containing the reference methyl esters was sprayed with the visualizing agent 0.1 % 2', 7'-dichlorofluorescein with methanol. Under ultraviolet light the reference methyl esters appear as two overlapping spots or bands. The upper band contained the more saturated while the lower containing the more unsaturated methyl esters. The portion of the plate pointing the methyl esters was marked and the silica was scraped off from the plate into a test tube. 7 ml of chloroform was added to methyl esters and the silica in the test tube. It was filtered through a Pasteur pipette containing the glass wool for removing the silica. After this treatment, the purified water and hexane were added and the upper organic layer was transferred to a vial. This step was performed several times to achieve the complete extraction of FAME (Bligh & Dyer ,1959). Samples were then dried and dissolved again in 20 ul hexane to get 50 times concentration and also to remove all solvent peaks (toluene). The concentrated samples were then injected into gas chromatography–mass spectrometry to read the spectra using caprylic acid ($CH_3(CH_2)_6COOH$) as an internal standard (Zaleha *et al.*, 2014). Similarly, the wild harpacticoid species from the Puzhal Lake were isolated and the same procedure was repeated for the fatty acid analysis. The percentage of fatty acids detected from the cultured and wild samples were expressed in mean and standard deviation.

III. RESULTS

The fatty acid analysis of harpacticoid species collected from the wild showed the presence of 24 fatty acids (Table: 1). The highest percentage was shown by nonadecanoic acid, methyl ester (2.25 ± 0.27 %), followed by methyl 6,9,12-hexadecatrienoate (1.88 ± 0.50 %) and 4,7,10,13,16,19-docosahexaenoic acid, methyl ester, (all-Z)- (1.63 ± 0.29 %) and the lowest was shown by 15-tetracosenoic acid, methyl ester, (Z)-, tetracosanoic acid, methyl ester and 5,8,11,14,17-eicosapentaenoic acid, methyl ester, (all-Z)- (0.04 ± 0.05 %).

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In cultured sample, 26 fatty acids were present and the highest percentage was obtained by hexadec-9-enoate (2.21 ± 0.13 %), followed by 5,8,11,14,17-eicosapentaenoic acid, methyl ester (1.85 ± 0.01 %) and hexadecanoic acid, methyl ester (1.83 ± 0.21 %) and the lowest percentage was shown by heptadecanoic acid,16-methyl-, methyl ester (0.05 ± 0.02 %) (Table: 1).

Table 1: Fatty acids of harpacticoid (*O. mohammed*) samples

S.no	Compound	Result (%)	
		Wild	Culture
1	Tridecanoic acid, 12-methyl-, methyl ester ($C_{15}H_{30}O_2$)	0.99±0.02	1.21±0.12
2	1- Tetradecanol ($C_{14}H_{30}O$)	-	0.16±0.05
3	Pentadecanoic acid, methyl ester ($C_{16}H_{32}O_2$)	0.15±0.04	0.18±0.08
4	Methyl 6,9,12,15-hexadecatetraenoate ($C_{17}H_{26}O_2$)	0.44±0.09	0.07±0.01
5	Methyl 6,9,12-hexadecatrienoate ($C_{17}H_{28}O_2$)	1.88±0.50	0.42±0.13
6	Methyl hexadec-9-enoate ($C_{17}H_{32}O_2$)	1.59±0.51	2.21±0.13
7	Hexadecanoic acid, methyl ester($C_{17}H_{34}O_2$)	0.08±0.42	1.83±0.21
8	6-Hexadecenoic acid, 7-methyl,methyl ester (Z) ($C_{18}H_{34}O_2$)	0.20±0.13	0.11±0.01
9	tert-Hexadecanethiol ($C_{16}H_{34}S$)	-	0.09±0.02
10	Hexadecanoic acid, 14-methyl-, methyl ester($C_{18}H_{36}O_2$)	-	0.23±0.06
11	gamma.-Linolenic acid, methyl ester($C_{19}H_{32}O_2$)	-	0.46±0.07
12	Heptadecanoic acid, methyl ester ($C_{18}H_{36}O_2$)	0.43±0.09	0.05±0.01
13	Methyl stearidonate ($C_{19}H_{30}O_2$)	0.38±0.04	0.46±0.02
14	12,15-Octadecadienoic acid, methyl ester ($C_{19}H_{36}O_2$)	1.18±0.07	1.65±0.09
15	9-Octadecenoic acid, methyl ester, (E)- ($C_{19}H_{36}O_2$)	0.85±0.06	-
16	11-Octadecenoic acid, methyl ester($C_{19}H_{36}O_2$)	0.67±0.15	1.04±0.12
17	Heptadecanoic acid,16-methyl-, methyl ester ($C_{19}H_{38}O_2$)	0.08±0.04	0.05±0.02
18	Nonadecanoic acid, methyl ester ($C_{20}H_{40}O_2$)	2.25±0.27	0.09±0.03
19	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- ($C_{21}H_{32}O_2$)	0.04±0.05	1.85±0.01
20	5,8,11,14-Eicosatetraenoic acid, methyl ester ($C_{21}H_{34}O_2$)	-	0.50±0.01
21	Methyl 8,11,14-heptadecatrienoate ($C_{18}H_{30}O_2$)	0.24±0.07	0.20±0.04
22	cis-11-Eicosenoic acid, methyl ester ($C_{21}H_{40}O_2$)	0.14±0.10	0.15±0.06
23	Methyl 18-methylnonadecanoate ($C_{21}H_{42}O_2$)	0.38±0.05	0.40±0.09
24	Methyl 6,9,12,15,18-heneicosapentaenoate ($C_{22}H_{34}O_2$)	0.14±0.02	-
25	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)- ($C_{23}H_{34}O_2$)	1.63±0.29	1.34±0.19
26	Methyl 7,10,13,16,19-docosapentaenoate ($C_{23}H_{36}O_2$)	0.46±0.03	0.31±0.11
27	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester ($C_{23}H_{34}O_2$)	-	1.34±0.42
28	Methyl 7,10,13,16,19-docosapentaenoate ($C_{23}H_{36}O_2$)	-	0.31±0.03
29	Docosanoic acid, methyl ester ($C_{23}H_{46}O_2$)	0.08±0.05	-
30	15-Tetracosenoic acid, methyl ester, (Z)- ($C_{25}H_{48}O_2$)	0.04±0.01	-
31	Tridecanoic acid, 12-methyl-, methyl ester ($C_{15}H_{30}O_2$)	0.04±0.02	-

The fatty acids such as 1-tetradecanol, tert-hexadecanethiol, hexadecanoic acid, 14-methyl-, methyl ester, gamma.-linolenic acid, methyl ester and cis-5,8,11,14,17-eicosapentaenoic acid were obtained from cultured harpacticoid sample. But, the fatty acids that are present in wild harpacticoids *viz.*,tridecanoic acid, 12-methyl-, methyl ester,12,15-octadecadienoic acid, methyl ester, 9-octadecenoic acid, methyl ester, (E)-, 11-octadecenoic acid, methyl ester and tetracosanoic acid, methyl ester fatty acids were not found from cultured *O. mohammed*.

The omega – 3 fatty acids that are essential for fish larvae *viz.*, gamma-linolenic acid, methyl ester, 5,8,11,14-eicosatetraenoic acid, methyl ester, 5,8,11,14,17 eicosapentaenoic acid, methyl ester, cis-5,8,11,14,17-eicosapentaenoic acid and 4,7,10,13,16,19-docosahexaenoic acid, methyl ester were found to be present in cultured *O. mohammed*.

IV. DISCUSSION

Generally, harpacticoid copepods in wild are known to posses the essential fatty acids and it is important to know the nutritional aspect of the cultured harpacticoids. The results obtained from fatty acid analysis indicate slight variation in the fatty acid composition of cultured *O. mohammed* from that of the wild sample. It contains diverse lipid compounds of carbon ranging between C_{14-25} in both the samples. Among them, C_{17} and C_{19} occurred in maximum percentage. The environmental conditions and the diet play a vital deciding factor for the synthesis of fatty acids (Zaleha *et al*., 2014). So, the fatty acid profile will not be constant and this supports the results of the current study. The omega 6 fatty acids like gamma-linolenic acid, methyl ester (18 : 3 (n - 6)) γLnA ; 5,8,11,14- eicosatetraenoic acid, methyl ester, (20 : 4 (n - 6)) ARA and omega 3 fatty acids such as cis-5,8,11,14,17-eicosapentaenoic acid (20 : 5 (n-3)) EPA and 4,7,10,13,16,19-docosahexaenoic acid, methyl ester (22 : 6 (n - 3)) DHA are the essential fatty acids and that were present in the cultured harpacticoids.

The most occurring essential fatty acid in the cultured sample of *O. mohammed* was EPA and DHA. The consumption of EPA and DHA has shown to have more health benefits. The essential fatty acids like EPA, DHA and ARA are required for the zooplankton, fish and humans. They promote growth, reproduction and neural development in aquatic animals and for human they take part in anti-inflammation and neural responses (Jans *et al.*, 2010; Ladhar *et al*., 2014). High consumption of long chain omega 3 fatty acids might reduce incidence of heart disease and reductions in total mortality (Hu *et al*., 2003). DHA comprises one third of the total fatty acid composition of the retina, cerebellum and cerebrum (Neuringer, 1988) and it is also required for optimal cognitive and visual development.

Zaleha *et al*. (2014) observed the higher expression of fatty acids with rich DHA and EPA at optimum condition (pH 7 and salinity 25 to 30 ppt) in *Pararobertsonia* sp. Goncalves *et al.*, 2012, reported diverse and rich fatty acids in copepods during winter and spring seasons. In another experiment, *Parastenhelia* sp. was checked for its fatty acid composition at various temperatures and pH and found higher EPA, DHA and ARA at pH 8 ± 0.3 (Jayalakshmi *et al*., 2016). These experiments set an example for the influence of various environmental factors on the fatty acid composition of harpacticoids as shown in the present study.

In the present study, the important fatty acids like DHA 22 : 6 (n - 3), EPA 20 : 5 (n - 3), ARA 20 : 4 (n - 6) and gamma linolenic acid 18 : 3 (n - 6) are recorded as 1.34 ± 0.19 %, 1.85 ± 0.01 %, 0.50 ± 0.01 % and 0.46 ± 0.07 % respectively. The fatty acid acid content of species varies for instance, *Tigriopus californicus* found to contain 5.3 % of EPA and 13.5 % of DHA when fed with commercial fish flakes along with *Isochrysis galbana* (Kreeger *et al*., 1991). Watanabe *et al*., (1978) found that *Tigriopus* sp. contains high level of highly unsaturated fatty acid nearly 12 % of DHA and 7 % of EPA. De Lima *et al*., (2013) cultured *Tisbe biminiensis* and found that the mixed diet fed culture produced abundant essential fatty acids such as EPA and DHA as 1.3 % and 3.3 % correspondingly. *Tisbe furcata* fed with *Rhodomonas* sp. was found to produce EPA and DHA as 13.1 % and 7.2 % respectively (Nanton & Castell, 1999).

Amonardia sp. cultured with microalgae was estimated to have 0.2 ± 0.1 to 2.7 ± 0.8 of EPA, 8.8 ± 2.6 to 18.5 ± 2.9 of DHA and 0.3 ± 0.1 of ARA (Nanton & Castell, 1999). *Nitokra lacustris* found to contain 2 % of EPA, 8 % of DHA 0.4 % of ARA when fed with *Tetraselmis suecica*, a green alga but the same harpacticoid fed with formulated feed was found to contain 3 % of EPA, 1.1 % of ARA and same quantity of DHA (Rhodes & Boyd, 2005). The presence of DHA and EPA of *O. mohammed* is comparatively lower than the above mentioned harpacticoids. Quantity of EPA (1.85 ± 0.01 %) and DHA (1.34 ± 0.19 %) of *O. mohammed* satisfies the optimum requirement for fish larvae which is stated as more than or equal to 1 % (Sargent *et al*., 1997). Therefore *O. mohammed* can be regarded as a suitable species for feeding the finfish and shellfish larvae in aqua hatcheries.

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