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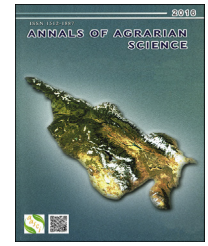

Effect of acidification on fatty acids profiling of marine benthic harpacticoid copepod *Parastenhelia* sp.

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ABSTRACT

Effect of acidification on the fatty acid (FA) profile of benthic harpacticoid copepod *Parastenhelia* sp. was investigated in-vitro. Copepods were exposed to different pH viz. 4 ± 0.3, 5 ± 0.3, 6 ± 0.3, 7 ± 0.3 and 8 ± 0.3 at constant salinity of 32‰ and a temperature of 28 °C for 30 days. After the experiment, fatty acid profiles of test and control copepods (pH: 8 ± 0.3) were analysed using Gas Chromatography and Mass Spectrometry (GC–MS). The present study reveals the negative influence of acidification on the fatty acid contents in copepods. The detected FAs are ranging from C4–C24. Among the fatty acids, Heneicosanoic acid and cis–11, 14–eicosadienoic acids are found in higher percentage in all the pH levels. However, highly unsaturated fatty acids such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) are reported to be low in acidified pH of 4 ± 0.3 compared to other pH tested. Results clearly indicate that the acidification can adversely affect the fatty acids profile of marine copepod *Parastenhelia* sp. that can be considered as one of the candidates for live feed of fish and shrimp for larval production in wild and aquaculture industry.

Introduction

It is well known that pH is playing a vital role in aquaculture as a measure of the acidity of the water or soil. If pH changes considerably, its make shrimp and fish shocked, weakened and stop eating. If high or low pH extends for a long time, it will make shrimp grow slowly, stunting and subject to diseases. Fish cannot survive in waters with acidified pH 4 and alkaline pH 11 for longer periods and also affect the growth, reproduction and denaturing the cellular membranes. The optimal pH for fish is between 6.5 and 9. Copepods are one of the most important natural food sources to the upper tropic level organism mainly marine fish larvae. Due to its wide range of sizes, high levels of protein, digestive enzymes and a rich source of fatty acids compared to commercial and conventional larval feeds (*Artemia* and Rotifers), they are preferentially used by the aquaculture industries as an initial feed for the early larval stages of fishes [3]. Many studies

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have reported that fish larvae fed with early stages of copepods attain improved survival, better growth and pigmentation [1,4,5,13,20,22]. This is generally due to the availability of high level of essential fatty acids such as, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and arachidonic acid (ARA) in copepods as key nutrients that influence food quality and also enhance somatic growth and reproductive rates of larval fish and invertebrates [21]. Studies have shown that most marine larval fish feeds on copepod eggs and different stages of nauplii during the first few weeks until exogenous feeding [15]. Several studies stated that ocean acidification tends to reduce the fatty acid composition of copepods [11,25]. Hence, the present study is aimed at investigating the effect of pH on the fatty acid (FA) profile of benthic harpacticoid copepod *Parastenhelia* sp. *in-vitro* condition.

Materials and methods

Sample collection and acclimation

Copepod samples were collected from the Point Calimere coastal waters (Lat. 10°15°21 N; Long. 78°48°50 E), Southeast Coast of India, during early in the morning by using plankton net with 158 μm mesh by horizontal towing. The collected samples were immediately transported to the laboratory provided with sufficient aeration by using battery aerators and the samples were thoroughly rinsed to reduce the contamination from other zooplankters. At laboratory, the species was identified using the morphological characters by referring the article [23]. The stock culture of *Parastenhelia* sp. was maintained following the standard method [19] and acclimated in indoor culture condition at temperature 28 °C and 32‰ salinity, fed with *Isochrysis galbana*.

Experimental setup

A total of 200 healthy adult individuals of *Parastenhelia* sp. were transferred from stock culture to serially arranged glass bowls (250 ml) covered using black chart to prevent light penetration. Water quality parameters were maintained as at constant temperature of 28 °C, salinity of 32‰ and exposed to different pH (4 ± 0.3, 5 ± 0.3, 6 ± 0.3±0.3, 7 ± 0.3 and control 8 ± 0.3) in triplicates. During the experiment, algal concentration was maintained at 0.18 μg/l. Salinity was measured using hand refractometer (Atago, Japan) and pH using Elico pH meter (Model LC-120). Percentage mortality of experimental copepods was recorded every 3 days interval (mortality ranged between 10% and 60%). After 30 days of experimental period, the samples were oven-dried for fatty acid analysis. The extraction of fatty acids in control and test copepods was made according to AOAC [2]. Identification and quantification of fatty acids were done by using a Gas Chromatography and Mass Spectrometry (Hewlett Packard 5890 Model).

Statistical analysis

Data were analysed by one-way ANOVA. Tuckey's test was used to rank the groups. Data are presented as mean ± S.D. All statistical analyses were performed using Graph Pad Prism Version 6.

Results

Wide ranges of essential fatty acids (EFAs) and non-essential fatty acids (nEFAs) were detected in different pH treatments with the carbon numbers ranging from C_{4-24} with a significant difference at $p < 0.05$. Our results showed the quantitative and qualitative expressions of fatty acids increased in pH 8 ± 0.3 (alkaline), compared to buffer and acidic 7 ± 0.3, 6 ± 0.3, 5 ± 0.3 and 4 ± 0.3 (acidic) treatments. EFAs such as *cis*-11, 14, 17-eicosatrienoic acid and *cis*-8,11,14-eicosatrienoic acid contributed in lower percentage (0.40–1.25%) while *cis*-11, 14-eicosadienoic acid (9.10–17.30%) followed by Linolenic acid (4.24–16.26%) were comparatively in higher levels in different pH levels. Generally highly unsaturated fatty acids such as EPA, DHA and Arachidonic acid were higher in control pH (8 ± 0.3) while lower in acidic pH (4 ± 0.3) resulted 0.04–13.71%. The Erucic acid was found ranged from 2.94 to 9.40% and *cis*-11-Eicosenoic acid was found only in pH 6 ± 0.3, 7 ± 0.3 and 8 ± 0.3 (0.46–5.51%) (Table 1).

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

Group	Common names	Samples				
		pH: 4 ± 0.3	pH: 5 ± 0.3	pH: 6 ± 0.3	pH: 7 ± 0.3	pH: 8 ± 0.3
EFA	Linolenic acid (C18)	4.24 ± .69^{a}	10.73 ± .08^{b}	13.53 ± .79^{c}	14.41 ± .60^{c}	16.26 ± 1.23^{d}
	cis-11-Eicosenoic	–	–	0.46 ± 0.20^{a}	0.53 ± 0.13^{a}	5.51 ± 0.71^{b}
	cis-11,14-eicosadienoic	9.10 ± 0.50^{a}	14.69 ± 0.81^{b}	15.39 ± 0.92^{bc}	16.64 ± 1.12^{cd}	17.30 ± .47^{d}
	cis-8,11,14-eicosatrienoic	0.40 ± 0.10^{ab}	0.36 ± 0.08^{a}	0.63 ± 0.05^{abc}	0.65 ± 0.09^{bc}	0.80 ± 0.28^{c}
	Erucic acid (C22)	2.94 ± 0.67^{a}	8.34 ± 1.13^{b}	8.62 ± 0.65^{b}	9.10 ± 0.44^{b}	9.40 ± .61^{b}
	cis-11,14,17-eicosatrienoic	0.98 ± 0.02^{a}	1.10 ± 0.17^{a}	1.19 ± 0.13^{a}	1.20 ± 0.22^{a}	1.25 ± .18^{a}
	Arachidonic acid (C20)	2.29 ± 0.59^{a}	3.91 ± 0.22^{b}	4.55 ± 0.59^{bc}	4.93 ± 0.61^{c}	5.15 ± .53^{c}
	Eicosapentaenoic acid (EPA)	0.04 ± 0.02^{a}	0.14 ± 0.05^{a}	1.38 ± 0.15^{b}	10.97 ± 0.13^{c}	11.98 ± .82^{d}
	Docosahexaenoic acid (DHA)	0.88 ± 0.60^{a}	5.56 ± 0.30^{b}	9.21 ± 0.18^{c}	11.09 ± 0.03^{d}	13.71 ± .63^{e}

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

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