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NOTES

Fatty Acid Composition of *Daphnia pulex* Cultured by Two Different Methods

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Freshwater zooplankton, such as *Daphnia,* serve as an important live food organism in the culture of numerous larval fishes (De Pauw et al. 1981). Ogino (1963) stated that various species of zooplankton are valuable protein sources and have similar amino acid compositions. Success of *Daphnia* culture is frequently determined by choice of food (De Pauw et al. 1981; Langis et al. 1988). Diet has been shown to influence the nutritional value of the cultured organism (Hinchcliffe and Riley 1972; Watanabe et al. 1983) and, therefore, live food organisms grown on different food sources or media might have different nutritional qualities for larval fishes (Walford and Lam 1987; Kissil and Koven 1990).

Several species of fish larvae require highly unsaturated fatty acids (HUFA) of the n-3 series in their diet (Cowey et al. 1976; Yingst and Stickney 1979; Kanazawa et al. 1982; Levine and Sulkin 1984). Fatty acid composition of zooplankton is influenced by the fatty acid composition of their food source (Hinchcliffe and Riley 1972; Watanabe et al. 1983) and the effectiveness of *Daphnia* as a food organism may be principally related to its diet.

When fish are reared in ponds, zooplankton populations are successfully managed by addition of organic and inorganic fertilizers, inoculation of rearing ponds with desired zooplankton species, and stocking defined numbers of larval fish per hectare (Geiger 1983). However, in a hatchery, fish are often fed live foods cultured indoors (Buddington and Doroshov 1984). The objective of this study was to determine if two different culture methods, pond-rearing and indoor-rearing (using a bacterial medium), affect fatty acid composition of *Daphnia pulex.*

This study was conducted at the Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky. Indoor culture of *Daphnia pulex* was conducted in two 3.0 m^3 fiberglass tanks containing dechlorinated tap water. Fresh horse manure was introduced into the water via floating, screen-bottom boxes (0.6 × 0.6 m) according to a rate (1.67 kg manure/m^3 initial) and schedule (0.75 kg manure/m^3 once every week thereafter) recommended by Ivleva (1969). The tanks contained air-lift pumps that created a water flow rate of 2.0 L/min and that were run for oxygenation and to prevent stratification. Water temperature was maintained at 18 C in a temperature controlled room. *D. pulex* were inoculated at a rate of 5 g/m^3.

Pond-reared *D. pulex* were collected from a 0.04 ha earthen pond fertilized two times in February with a total of 4 L of liquid inorganic fertilizer (13-34-0; N-P-K). Water temperature was 10 C. Pond inoculation was not necessary because a natural population of *D. pulex* is prominent in the spring. After four weeks, separate 10 g collections of *D. pulex* were collected on 21 March with a Wisconsin-style plankton net (80 μm) from each of the two indoor culture tanks and two separate samples from the pond. Samples were examined with a dissecting microscope to verify that only *D. pulex* were present, and samples were frozen with liquid nitrogen.

Total lipids were extracted from approx-

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TABLE 1. *Percentage crude protein (dry weight), lipid (dry weight), and moisture of* Daphnia *collected from a pond or grown on a horse manure-based bacterial medium indoors. Values are means ± SE of two replications. No significant differences (P > 0.05) were found among treatments.*

	Daphnia grown in	
	Pond	Bacterial medium
Crude protein (%)	65.6 ± 8.5	66.8 ± 2.6
Crude lipid (%)	23.6 ± 0.6	20.9 ± 3.8
Moisture (%)	91.2 ± 0.6	89.8 ± 0.3

imately 2 g of each sample according to procedures described by Kates (1986). Fatty acid methyl esters were obtained by following the methods described by Metcalfe et al. (1966). Separation of fatty acid methyl esters was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a 30 m fused-silica capillary column DB225 (J&W Scientific, Folsom, California) and a flame-ionization detector. Identification of individual fatty acids was accomplished by comparison of their retention times with those of authentic standards (Nu-Chek Prep, Inc., Elysian, Minnesota).

Crude protein was determined using a LECO FP-228 nitrogen determinator (Sweeney and Rexroad 1987). Total lipid and moisture were determined according to methods of AOAC (1980). Student's *t*-test was used to compare means. Percentage data were normalized by arcsine transformation prior to statistical analysis.

Percentage crude protein, lipid, and moisture were not significantly different in *D. pulex* from the two treatments ($P > 0.05$) (Table 1). Pond populations of *D. pulex* had significantly higher ($P < 0.05$) percentages of the n-3 fatty acids 18:3(n-3), linolenic acid, and 20:5(n-3), eicosapentaenoic acid (EPA), than *D. pulex* cultured in bacterial medium (Table 2). Total percentage of n-3 fatty acids was significantly higher ($P < 0.05$) in *D. pulex* collected from ponds (27.4%) compared to those grown in bacterial medium (10.5%).

The reduced levels of n-3 HUFA in *D.*

TABLE 2. *Percentage of total weight (area percent) of selected fatty acids in* D. pulex *collected from a pond or grown indoors in a horse manure-based bacterial medium. Values are means ± SE of two replications. Means within the same row with an asterisk "*" are significantly different (P < 0.05).*

	Daphnia grown in	
Fatty acid	Pond	Bacterial medium
12:0	0.0 ± 0.0	0.1 ± 0.0
13:0	0.0 ± 0.0	0.1 ± 0.0
14:0	1.7 ± 0.1	1.7 ± 0.2
14:1 (n-5)	0.0 ± 0.0	0.1 ± 0.0
15:0	0.7 ± 0.0	1.7 ± 0.3*
16:0	14.7 ± 0.3	12.9 ± 0.3
16:1 (n-9)	1.3 ± 0.1	1.4 ± 0.2
16:1 (n-7)	6.7 ± 0.2	13.2 ± 0.1*
16:1 (n-5)	0.6 ± 0.2	1.3 ± 0.0
16:2 (n-7)	0.0 ± 0.0	0.1 ± 0.0
16:2 (n-4)	0.3 ± 0.1	0.0 ± 0.0
16:3 (n-4)	0.3 ± 0.1	0.2 ± 0.1
16:3 (n-3)	1.0 ± 0.2	0.0 ± 0.0*
16:4 (n-1)	1.1 ± 0.3	0.3 ± 0.0*
17:0	2.1 ± 0.2	1.9 ± 0.1
18:0	3.5 ± 0.2	6.3 ± 0.1*
18:1 (n-9)	12.1 ± 0.4	12.6 ± 0.1
18:1 (n-7)	5.4 ± 0.1	9.5 ± 0.1*
18:1 (n-5)	0.0 ± 0.0	0.4 ± 0.0
18:2 (n-6)	5.1 ± 0.1	4.9 ± 0.0
18:2 (n-4)	0.6 ± 0.3	0.9 ± 0.1
18:3 (n-3)	12.2 ± 0.4	2.6 ± 0.1*
18:4 (n-3)	1.8 ± 0.3	0.7 ± 0.2*
18:4 (n-1)	0.4 ± 0.1	0.2 ± 0.2
19:0	0.5 ± 0.1	0.0 ± 0.0
20:3 (n-6)	0.1 ± 0.0	0.4 ± 0.0
20:4 (n-6)	3.3 ± 0.4	9.7 ± 0.6*
20:4 (n-3)	0.4 ± 0.0	0.0 ± 0.0
20:5 (n-3)	10.3 ± 0.7	5.7 ± 0.1*
22:5 (n-6)	0.0 ± 0.0	0.3 ± 0.0
22:5 (n-3)	0.4 ± 0.1	0.0 ± 0.0
22:6 (n-3)	1.4 ± 0.2	1.6 ± 0.1
24:0	0.7 ± 0.1	0.2 ± 0.1
Identified fatty acids[a]	91.2	94.1
Unknown	8.8 ± 0.7	5.9 ± 0.0
% saturated	23.8 ± 0.5	24.7 ± 0.6
% monoene	26.4 ± 0.0	38.7 ± 0.1*
% diene	5.9 ± 0.3	5.9 ± 0.0
% polyene	35.1 ± 1.3	24.8 ± 0.1*
% n-3	27.4 ± 1.3	10.5 ± 0.1*
n-3/n-6	3.2 ± 0.1	0.7 ± 0.0*

[a] Total value includes iso 15:0 and 17:0 which are not presented in the table.

pulex grown in a bacterial medium agree with findings of James and Abu-Rezeq (1989) who reported that rotifers cultured indoors had a reduced n-3 HUFA content, especially EPA. It appears that wastewater-grown bacteria, and thereby the organisms that utilize them as a food source, are not rich in n-3 HUFA (Watanabe et al. 1978; Proulx and de la Noüe 1985). The lower percentage of linolenic acid and EPA in *D. pulex* cultured in a bacterial medium is inadequate for feeding marine fish larvae based upon the levels recommended by Watanabe et al. 1983.

Daphnia have been cultured on different food sources and their nutritional requirements reported (Taub and Dollar 1968; De Pauw et al. 1981; Langis et al. 1988). This study indicates that *D. pulex* collected from a pond in March have a high percentage of n-3 HUFA. However, fatty acid composition may change as the seasonal succession of phytoplankton species in the pond occurs (Jeffries 1970). Proulx and de la Noue (1985) reported that fatty acid composition of *D. magna* grown on *Scendesmus* sp. contained no n-3 HUFA. Thus, the nutritional value of pond-cultured *D. pulex* may not always be superior to that of *D. pulex* cultured in a bacterial medium. Rainuzzo et al. (1989) increased n-3 HUFA in rotifers, *Brachionus plicatilis,* by feeding the rotifers on algae containing high levels of n-3 HUFA. Enhancement of n-3 HUFA levels in *D. pulex* cultured in a bacterial medium could be achieved by additionally feeding certain species of algae (*Bacillophyceae*), or oil emulsions with high HUFA contents.

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