Nutritional value of freshwater mesozooplankton assemblages from Hanna Dam Lake, Iran, during a one-year study

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Abstract

Nutritional value of freshwater mesozooplankton, fatty acid (FA) and amino acid (AA) compositions were determined in the middle of each season for a one-year period from May 2009 to February 2010 in Hanna Dam Lake, Isfahan, Iran. FA and AA composition significantly ($P<0.05$) varied in relation to the seasonal changes of water quality, phytoplankton and zooplankton community. The content of saturated fatty acids (SAFA), mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranged from 22.4-29.0%, 30.9-40.4%, 11.8-20.9 % of dry weight (DW), respectively. The major SAFA were 16:0 (13.7-17.0 % DW) and 18:0 (4.9-7.0 % DW), whereas contents of MUFA were mainly 18:1n-9 (13.8-16.2 % DW), 16: 1n-7 (6.9-13.6% DW), and 18:1n-7 (5.7-10.6% DW). The major PUFA were 18: 2n-6 (2.6-11.7 % DW), 18: 3n-3 (2.4-3.1% DW), 20: 5n-3 (3.9-4.8% DW), 22: 6n-3 (0.73-0.99% DW), and 20: 4n-6 (0.56-0.73% DW). As for the ratios of n-3:n-6, the values were 0.70:1, 2.54:1, 2.10:1, and 1.73:1 in spring, summer, autumn and winter respectively. The mean essential amino acid (EAA) and non-essential amino acid (NEAA) were 28.7 and 71.3 %; 31.0 and 69.0 %; 31.63 and 68.4 %; 34.5 and 67.0 % of total amino acid in spring, summer, autumn and winter, respectively. The amount of tyrosin, isolucine, lucine, arginine, cysteine, aspartic acid, glycine and proline were higher in mesozooplankton population at summer and autumn compared to winter and spring.

Keywords: Nutritional value, Zooplankton, Seasonal variability, Eutrophic Lake, Iran

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Introduction

Zooplankton is very important group of living organisms, as they are the basis for food web in aquatic ecosystems. Among zooplankton, cladocerans and copepods play a key role in the freshwater food chains. They transfer energy from microalgae to higher trophic levels by being eaten as a food through various aquatic organisms (Goldman and Horne, 1983; Ahlgren et al., 1990; Harris et al., 2000).

These organisms contain a valuable source of lipid and fatty acids (FAs), protein and amino acids (AAs), vitamins and enzymes (Pillay, 1990; Izquierdo et al., 2000; Evjemo et al., 2001). Owing to FA and AA importance of zooplankton in food web, comprehensive studies carried out on their nutritional values (Ahlgren et al., 1996; Adams, 1999; Ballantyne et al., 2003; Muller-Navarra et al., 2004; Brett et al., 2006). In addition to the work on FAs and AAs in zooplankton, extensive information about their origin and fate in zooplankton and their physiological status has been provided (Mourente, 2003; Mitra et al., 2007; Boechat and Giani, 2008; Guo et al., 2008; Kainz et al., 2009). It has been demonstrated that several environmental factors such as temperature, depth, salinity, geographical location, season, sex, molting status, developmental stage, food availability, photoperiod, stress and starvation could affect the FAs and AAs profile of zooplankton (Gardner and Riley, 1972; Graney and Giesy, 1986; Narasimmalu et al., 1991).

Although there are many reports on nutritional values of different zooplankton groups from separated geographical location (Graney et al., 1986; Kibria et al., 1999; Kainz et al., 2004; Mitra et al., 2007; Boechat and Giani, 2008), information on nutritional values of mixed zooplankton from Iranian aquatic ecosystems is scarce. The main purpose of this study was to provide basic information on FA and AA composition of mixed freshwater mesozooplankton assembelages from Hanna Dam Lake situated at Eastern part of Isfahan province, Iran (Fig. 1) during different seasons. Furthermore, this paper will deal with the influence of accessory factors, such as water quality, phytoplankton and zooplankton community structure on FA and AA composition of mesozooplankton assemblages during a one-year. Findings of this study could be used as nutritional values of zooplankton community for its ecological and biological properties in freshwater ecosystems.

Materials and methods

Study area

The present study was carried out in the Hanna Dam Lake (latitude=31º 13’ - 31º 14’ N; longitude=52º 46’- 52º 47’ E; altitude=2300 m from sea level; area=700 ha; mean depth=10 m; average annual rain =380 mm), located in Eastern part of Isfahan province, Iran (Fig. 1). The region has a cold temperate climate with a minimum average temperature of -1.1◦C in February and 22.9 °C in August. The lake has an ecological importance for migratory birds, wildlife and aquatic organisms especially fish. Shallow parts of the lake are occupied by various species of emergent plants such as *Phragmites*, *Typha*, *Juncus* and *Cyperus*. Submerged

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plants such as *Potamogeton* spp., *Ceratophyllum demersum*, *Myriophyllum* sp. and *Polygonum amphibium* also exist in the lake from May to October. Hanna Lake has three indigenous fish species including, *Alburnus* sp., *Capoeta damascina* and *Aphanius isfahanensis* and two non-indigenous species of *Oncorhynchus mykiss* and *Carassius auratus*.

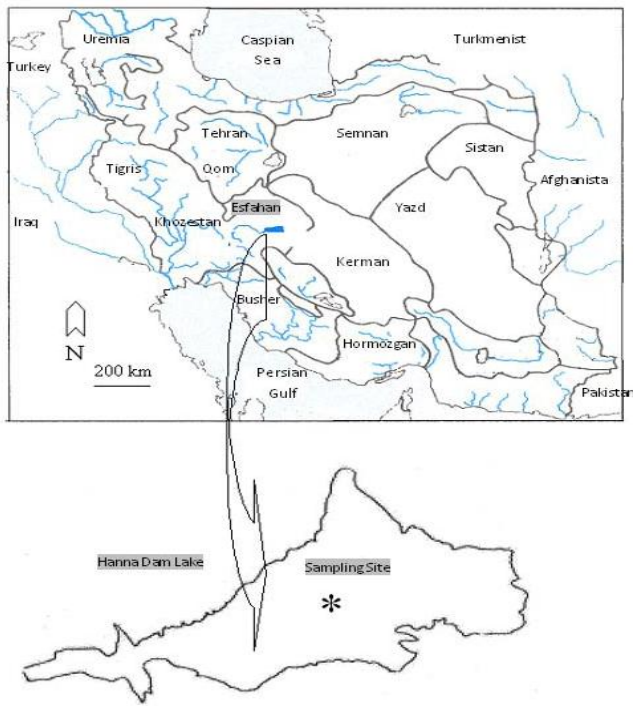


Figure 1: Location of study area and sampling site in Hanna Dam Lake, Isfahan, Iran.

Field sampling

Seasonal field samplings were carried out at middle of each season for a one-year period from May 2009 to February 2010. 3-liter water samples were collected from the water column at sampling site (Fig. 1) by a Van Dorn water sampler. Measurements were made of water temperature, Secchi depth, dissolved oxygen (YSI 51 Oxygenmeter, OH, USA), pH (WTW 330 pHmeter, Weilheium, Germany), TDS and Ec (HQ40d, Hach-Lange Company) *in situ*. Nutrients such as nitrate-nitrogen, ammonium-nitrogen and phosphorus were determined according to standard method (APHA, 1995) in Aquatic Fishery Laboratory at Isfahan University of Technology (AFL-IUT), Isfahan, Iran.

The zooplankton samples were collected between 10.00 and 12.00 h by vertical haul using zooplankton net (bolting silk, 140 µm mesh size, and diameter of 25 cm) from sampling site (Fig. 1). To obtain enough zooplankton biomass for nutritional analysis, the collected mixed zooplankton were transferred to 2-L cylindrical container and first three sub-samples of 30 mL was

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immediately fixed in 5% formaline for identification and quantitative estimation of relative density (abundance) of each zooplankton category. Then, rest of samples was filtered by 40-µm net and washed three times with filtered habitat water and chilled in -20 ºC for nutritional analysis.

For phytoplankton identification and estimation of abundance, the water samples collected from different depth (surface, middle, and bottom) and then mixed with same ratio. The Lugol's iodine solution (10 mL for 200 –mL sample) was used as fixative.

Phytoplankton and zooplankton

Phytoplankton samples were observed under phase contrast microscope and then identified by using literature of Davis (1955), Chaghtai and Salfullai (1988), Cox (1996), Clescceri et al. (1998), and Bellinger and Sigee (2010). Phytoplankton abundance was measured with a Sedgewick-Rafter counting chamber. Phytoplankton biovolumes were calculated using the literature of Bellinger and Sigee (2010). Chlorophyll *a* analysis was measured according to Wetzel and Likens (2000).

Zooplankton samples were initially identified to their major taxonomic groups of cladoceran and copepod. Since majority of mesozooplankton of present study were cladocerans and copepods, the adult individuals were separated from the sub-samples and preserved in small glass bottles using 70% ethyl alcohol for species identification. For identification of species literature of Ward and Whipple (1945), Edmondson (1959), Rylov (1963), Dussart (1965), Harding and Smith (1974), Harris et al. (2000), Schutze et al. (2000), Martin and Davis (2001), Fernando (2002) were used.

For estimation of zooplankton abundance, each sample was kept in measuring cylinder and adjusted to known volume by adding distilled water, and then transferred to a wide mouth 250 ml glass beaker. Next, a magnetic stirrer was set on the lowest speed for gently mixing of zooplankton sample and a sub-sample was taken using a Stemple pipette while mixing. Sub-sample was transferred to a zooplankton counting chamber (Bogorov's chamber) and zooplankton was counted under a dissecting microscope (Omori and Ikeda, 1984).

Nutritional measurements

Protein and lipid were determined following the method described by Meyer and Walther (1988). Fatty acid methyl esters (FAME) were prepared according to the direct methylation techniques (Divakaran and Ostrowski, 1989). A 100 mg of freeze-dried samples (in triplicate) were refluxed at 100 ºC for 10 minutes with 10 ml of 2% methanolic NaOH. The samples were further refluxed for 3 minutes with 6.25 ml 14% boron triflouride and another 2 minutes with heptane. After refluxing the samples were allowed to cool at room temperature. Then 2 ml of saturated NaCl was added (this made the FAME float). Then, the upper layer was removed and transferred into a centrifuge tube. One gram of anhydrous Na_2SO_4 was added to the collected samples to absorb the remaining water contained in the FAME. The mixture was then subjected to centrifugation at 3000

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rpm for 5 minutes. The supernatant was collected and stored in small glass tubes with tight cover inside the freezer until further analysis. The FAME of the samples were analyzed with a gas chromatograph (Shimadzu GC-8A) equipped with a FID and Supelco 2330 capillary column. The chromatograph used helium as the carrier gas. The column temperature was programmed from 150ºC to 190ºC at a ramp rate of 3ºC min^{-1}.The injector and detector temperatures were at 250ºC and 280ºC, respectively, while the BPX-70 temperature was maintained at 200ºC. The fatty acids were identified by comparison with retention times of known standards obtained from Sigma Chemicals Company (Ackman and Burgher, 1965). A Chromatopac (Shimadzu C-R3A, Japan) quantified the magnitude of the peaks of each chromatographic reading.

The sample preparation for determination of the amino acid profile consisted of: (1) hydrolysis of sample using 6N HCl and put in oven 110ºC for 24 hour; (2) add internal standard AABA (Alpha Amino Butric Acid) and then filtration; (3) dry sample and standard under vacuum for 30 minutes; (4) add re-drying solution (Metanol: Water: Triethylamine; 2:2:1) and then dry under vacuum for 30 minutes; (5) add derivation reagent (metanol: triethylamine: water: phenylisocyanate; 7: 1:1:1) and leave at room temperature for 20 minutes and then vacum for 30 minutes until dry. The quantification of amino acids was done by a reverse phase performance lipid chromatography system (Waters HPLC 501) equipped with a Bio-Rad prepared column (Pico. Tag column, 150 mm x 3.9 mm, USA) with methanol: tetrahyrosuran: 50 mM sodium acetate and 50 mM dibasic sodium phosphate pH= 6.8, and 65 % methanol as solvents. Amino acids were identified from retention time indices obtained by using Sigma amino acid standard. Amino acids results were expressed as weight percentage of total amino acids.

Statistical analysis

One-way ANOVA was performed to test for significant seasonal differences in FAs and AAs quantities, zooplankton density, and water quality parameters. Data are presented as means ± standard error of means. Differences in means were compared by Duncan's Multiple Range Test (Duncan, 1955). All percentage data were Arcsine-square root transformed and then tested for normal distribution and homogeneity of variance before performing ANOVA (Zar, 1984). All statistical analysis was carried out using Statistical Package for Social Science (SPSS 2002, version11.5).

Results

Water quality

Water quality parameters in the Hanna Dam Lake obtained in this study presented in Table 1. The water depth averages varied from 18.0 to 22.4m at sampling site, with the deepest at winter due to the high rainfall and high surface water inflow from surrounding area. The Secchi depth averages varied from 1.40 to 1.75 m and in autumn light penetration was higher compared to other seasons which may be supported better ecological/biological

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conditions for mesozooplankon in deeper layers. The mean water temperatures ranged 14.4-15.4, 19.9-21.3, 10.9-12.1, and 5.1-5.7 ºC in spring, summer, autumn and winter, respectively. The mean water dissolved oxygen (DO) and pH ranged 7.3-9.8 mg/L and 8.0-8.3, respectively. The nutrient concentrations were high in the lake. Concentrations of phosphorous ranged 0.03-1.20 mg PO_4/l , and highest concentration of NO_3-N was recorded 13.0 mg/l in winter. The ammonium-nitrogen concentrations varied from 0.45 to 1.62 mg/l with highest level in winter. The total dissolved solids (TDS) averages varied from 126.2 to 301.1 mg/l with the highest average at winter and spring due to the effects of high soil erosion loading from surrounding agricultural area.

Table 1: Water quality parameters during different seasons in Hanna Dam Lake, Isfahan, Iran. Shown are the mean based on 3 replicates.

		Spring	Summer	Autumn	Winter
Depth (m)		21.9	22.0	18.0	22.4
Secchi depth (m)		1.60	1.70	1.75	1.40
Air temp. (ºC)		18.80	25.20	15.91	11.97
Water temp. (ºC)	Sur.	15.40	21.27	12.09	5.73
	Mid.	14.60	20.73	11.0	5.10
	Bott.	14.40	19.94	10.92	5.20
Dissolved oxygen (mg/l)	Sur.	7.46	7.64	8.12	9.70
	Mid.	7.30	7.38	7.77	9.70
	Bott.	7.30	7.12	7.93	9.80
Nitrate (mg/l)	Sur.	1.08	1.04	1.95	8.90
	Mid.	1.32	1.10	1.93	12.60
	Bott.	1.08	1.05	1.80	13.00
Total phosphate (mg/l)	Sur.	0.051	0.97	0.67	0.031
	Mid.	0.043	1.22	0.54	0.030
	Bott.	0.063	1.15	0.67	0.030
pH	Sur.	8.10	8.24	8.17	8.2
	Mid.	8.10	8.28	8.13	8.2
	Bott.	8.00	8.19	8.12	8.2
Ammonium (mg/l)	Sur.	0.53	0.53	0.74	0.86
	Mid.	0.67	0.45	1.62	0.87
	Bott.	0.52	0.53	0.78	0.82
TDS (mg/l)	Sur.	283.9	129.7	148.3	299.5
	Mid.	289.8	126.2	156.3	301.1
	Bott.	284.3	128.1	147.9	297.6
EC (µScm-1)	Sur.	339.1	258.6	297.8	467.9
	Mid.	452.9	250.2	304.7	470.3
	Bott.	444.1	254.9	296.9	464.9

Surface (Sur.): 0-1 m; Middle (Mid.) = 10 m; Bottom (Bott.) =1m above bottom sediments

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Phytoplankton and zooplankton

The major groups of phytoplankton in this study were Chlorophyceae, Cyanobacteria, Bacillariophyceae, Dinophyceae and Desmidiaceae (Table 2). The dominant genera were *Staurstrum* and *Ceratium* in spring, *Surirella* and *Peridinium* in summer, *Ceratium* and *Staurstrum* in autumn, and *Staurstrum* and *Edorina* in winter. The relative biovolume of the Cyanobacteria was less than 1 % of the total phytoplankton in all seasons while Desmidaceae, except to summer, had highest percentage of biovolume in Hanna Dam Lake. The averages of chlorophyll *a* concentrations ranged from 2.55 to 9.43 mg/m^3, maximum in summer and minimum in winter.

Table 2: Relative abundance, relative biovolume, dominant genera of each phytoplankton group and chlorophyll *a* during the four seasons in Hanna Dam Lake, Iran

Seasons		Chlor.	Cyan.	Bacil.	Dino.	Desm.	Chl. *a* (mg/m^3)
Spring	Rel. abun%	65.73	17.21	7.48	0.56	9.03	8.25 ±2.24
	Rel. biov%	9.42	0.95	12.74	30.03	46.86	
		Gonium (5.5 %)	*Microcystis* (0.6 %) *Anabaena* (0.3 %)	*Surirella* (11.0 %) *Navicula* (1.0 %)	*Ceratium* (18.6 %) *Peridinium* (11.4 %)	*Staurastrum* (42.6 %) *Cosmarium* (4.3 %)	
Summer	Rel. abun%	64.29	12.70	18.25	1.32	3.44	9.43 ±1.42
	Rel. biov%	4.30 *Gonium* (2.1 %) *Chlorella* (1 %)	0.61 *Aphanothece* (0.3 %) *Microcystis* (0.3 %)	55.32 *Surirella* (55.1 %) *Synedera* (0.1 %)	30.16 *Peridinium* (30.2 %)	9.61 *Staurastrum* (9.6 %)	
Autumn	Rel. abun%	64.54	9.93	9.93	1.18	14.42	5.52 ±2.33
	Rel. biov%	17.39 *Eudorina* (14.3 %)	0.29 *Microcystis* (0. 2 %) *Aphanothece* (0.02 %)	11.06 *Surirella* (8.9 %) *Synedera* (1.8 %)	34.23 *Ceratium* (24.3 %) *Peridinium* (9.9 %)	37.03 *Staurastrum* (37.0 %)	
Winter	Rel. abun%	73.51	6.52	9.99	0.83	9.15	2.55 ±1.50
	Rel. biov%	29.73 *Eudorina* (25.4 %) *Gonium* (1.9 %)	0.09 *Aphanothece* (0.02 %) *Microcystis* (0.02 %)	14.69 *Surirella* (12.4 %) *Synedera* (1.7 %)	23.80 *Peridinium* (17.9 %) *Ceratium* (5.8 %)	31.69 *Staurastrum* (27.7 %) *Cosmarium* (4.0 %)	

Relative abundance (Rel. abun.), relative biovolume (Rel. biov), Chlorophyta (Chlor.), Cyanophycea (Cyan.), Bacillariophycea (Bacil.), Dinophycea (Dino.), Desmidacea (Desm.), Chlorophyll *a* (Chl. *a)*

Table 3 shows abundance and density of dominant species of mesozooplankton in Hanna Dam Lake during the study period. The total density of cladocerans was the highest in spring (47.62 ind./L) including four species of *Daphnia longispina*, *D. dubia*, *D. pulex* and *Moina macrocopa*. *D. longispina* were common species in lake.

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Bosmina sp. observed only in autumn may be due to short life cycle and higher consumption by fish larvae compared to small size cladoceran such as *Ceriodaphnia* and *Moina*.

The abundance of cladocerans and copepods in mixed zooplankton population were different (Table 3). The maximum abundance of cladocerans was observed in spring (74.37% in population) while the abundance of copepods were the highest in summer (72.52% in population) and autumn (72.77% in population).

Table 3: Zooplankton species, their seasonal abundance, and density in Hanna Dam Lake, Iran, Shown are the mean ± standard error based on 9 replicates

Zooplankton species		Spring	Summer	Autumn	Winter
Cladocera	*Daphnia longispina*	61.57	6.92	4.52	0.54
	Daphnia dubia	0.45	0.82	1.57	-
	Daphnia pulex	7.73	-	-	44.79
	Ceriodaphnia sp.	-	6.92	9.38	-
	Moina macrocopa	4.61	12.59	7.09	0.47
	Bosmina sp.	-	0.24	4.67	0.34
Cladocerans (% in population)		74.37 ^{a}	27.84 ^{c}	27.23 ^{c}	46.13 ^{b}
Copepoda	Metanauplii	-	-	2.07	46.61
	Copepodids	21.45	36.91	37.53	5.99
	Copepod (adults)	4.18	35.61	33.17	1.28
Copepoda (% in population)		25.63 ^{c}	72.52 ^{a}	72.77 ^{a}	53.87 ^{b}
Total zooplankton density (ind./L)		64.02 ^{b} ± 14.50	47.68 ^{c} ± 1.79	110.45^{a} ± 29.03	18.78 ^{d} ± 4.29
Cladoceran density (ind./L)		47.62 ^{a} ± 9.74	13.10 ^{c} ± 0.48	30.70 ^{b} ± 11.25	8.66 ^{d} ± 2.10
Copepods density (ind./L)		16.41^{c} ± 4.79	34.58 ^{b} ± 1.32	80.38 ^{a} ± 17.79	10.12 ^{d} ± 2.20

Identified species of copepods including: *Metacyclops* sp., *Acanthocyclops* sp., *Microcyclops varicans, Allocyclops* spp., *Diacyclops bicuspidatus, Macrocyclops albidus.* Means in the same row sharing a common superscript are not significantly different (P>0.05).

Nutritional values of mesozooplankton

The lipid and protein of mesozoplankton were 12.2 and 47.3 %; 13.4 and 54.3 %; 16.4 and 48.9 %; 15.6 and 42.1 % dry weight (DW) in spring, summer, autumn, and winter, respectively (Tables 4, 5). Seasonal FA composition of mixed zooplankton was tabulated in Table 4. The fatty acids of mixed zooplankton showed seasonal variation. The content of saturated fatty acids (SAFA), mono unsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranged from 22.4-29.0%, 30.9-40.4%, 11.8-20.9 % of dry weight (DW), in spring, summer, autumn and winter respectively. The major

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SAFA were 16:0 (13.7-17.0 % DW) and 18:0 (4.9-7.0 % DW), whereas contents of MUFA were mainly 18:1n-9 (13.8-16.2 % DW), 16: 1n-7 (6.9-13.6% DW), and 18:1n-7 (5.7-10.6% DW). The major PUFA were 18: 2n-6 (2.6-11.7 % DW), 18: 3n-3 (2.4-3.1% DW), 20: 5n-3 (3.9-4.8% DW), 22: 6n-3 (0.73-0.99% DW), and 20: 4n-6 (0.56-0.73% DW). As for the ratios of n-3:n-6, the values were 0.70:1, 2.54:1, 2.10:1, and 1.73:1 in spring, summer, autumn and winter respectively. The ratios of docosahexenoic acid (DHA, 22:6n-3) to eicosapentaenoic acid (EPA, 20:5n-3) of Hanna zooplankton ranged 0.18:1 to 0.23:1 in different seasons.

In this research, 11 essential amino acid (EAA) and 6 non-essential amino acid (NEAA) were determined in crustacean mesozooplankton assemblages of Hanna Dam Lake (Table 5). The mean EAA and NEAA were 28.7 and 71.3 %; 31.0 and 69.0 %; 31.63 and 68.4 %; 34.5 and 67.0 % of total amino acid in spring, summer, autumn and winter, respectively. Correspondingly, the mean EAA and NEAA were 94.8 and 235.7; 100.2 and 223.3; 115.2 and 248.9; 74.1 and 144.1 nmol/mg DW in spring, summer, autumn and winter, respectively.

During the entire year, glutamic acid (18.3-21.4%) represented the most abundant amino acid. The amount of tyrosin, isolucine, lucine, arginine, cysteine, aspartic acid, glycine and proline were higher in mesozooplankton population at summer and autumn compared to winter and spring (Table 5).

Table 4: Total lipid (% dry weight) and fatty acid composition (% dry weight) of mixed zooplankton collected from Hanna Dam Lake during study period Shown are the mean ± standard error based on 3 replicates.

	Spring	Summer	Autumn	Winter
Total lipid	12.25 ± 2.24 ^{b}	13.42 ± 0.95 ^{b}	16.41 ± 1.50 ^{a}	15.61 ± 3.20 ^{a}
14:0	2.42 ± 0.15 ^{b}	1.39 ± 0.10 ^{d}	2.04 ± 0.04 ^{c}	3.50 ± 0.10 ^{a}
14:1n-5	0.68 ± 0.16 ^{b}	0.96 ± 0.06 ^{c}	0.96 ± 0.02 ^{c}	1.97 ± 0.08 ^{a}
16:0	13.28 ± 0.02^{c}	13.72 ± 0.63 ^{c}	15.40 ± 0.31^{b}	17.02 ± 0.20^{a}
16:1n-7	8.30 ± 0.24 ^{c}	6.85 ± 0.43 ^{d}	10.54 ± 0.16 ^{b}	13.64 ± 0.34 ^{a}
17:0	0.90 ± 0.04 ^{c}	1.23 ± 0.07 ^{b}	1.39 ± 0.05 ^{a}	1.41 ± 0.19 ^{a}
17:1n-7	1.23 ± 0.04 ^{b}	1.25 ± 0.16 ^{b}	2.21 ± 0.16 ^{b}	2.79 ± 1.38 ^{a}
18:0	6.02 ± 0.44 ^{b}	5.92 ± 0.58 ^{b}	6.99 ± 0.09 ^{a}	4.94 ± 0.30 ^{c}
18:1n-9	16.20 ± 0.41 ^{a}	14.56 ± 0.72 ^{b}	14.25 ± 0.09 ^{b}	13.75 ± 0.58 ^{c}
18:1n-7	5.73 ± 0.08 ^{c}	6.87 ± 0.35 ^{b}	10.60 ± 0.21 ^{a}	7.84 ± 0.29 ^{b}
18:2n-6	11.65 ± 0.11 ^{a}	2.63 ± 0.38 ^{d}	3.70 ± 0.17 ^{c}	4.59 ± 0.42 ^{b}
18:3n-3	2.71 ± 0.05 ^{b}	3.11 ± 0.28 ^{a}	2.36 ± 0.03 ^{c}	2.79 ± 0.05 ^{b}
20:0	0.24 ± 0.01^{a}	0.14 ± 0.07 ^{b}	0.25 ± 0.01 ^{a}	-
20:1n-9	0.12 ± 0.06 ^{c}	0.43 ± 0.02 ^{a}	0.33 ± 0.02 ^{b}	0.43 ± 0.01^{a}
20:4n-6 (ARA)	0.62 ± 0.02 ^{b}	0.71 ± 0.13 ^{a}	0.56 ± 0.05 ^{c}	0.73 ± 0.02 ^{a}
20:3n-3	0.26 ± 0.01^{b}	0.22 ± 0.11 ^{c}	0.18 ± 0.09 ^{d}	0.30 ± 0.15 ^{a}
20:4n-3	0.44 ± 0.03 ^{b}	0.52 ± 0.05 ^{a}	0.29 ± 0.17 ^{c}	0.52 ± 0.05 ^{a}
22:0	-	-	2.04 ± 0.52	-
20:5n-3 (EPA)	4.41 ± 0.17 ^{b}	3.90 ± 0.28 ^{c}	4.31 ± 0.44 ^{b}	4.75 ± 0.23 ^{a}

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24:0	-	-	0.89 ± 0.24 [a]	0.25 ± 0.24 [b]
22:6n-3 (DHA)	0.79 ± 0.03 [c]	0.73 ± 0.04 [d]	0.99 ± 0.12 [a]	0.85 ± 0.18 [b]
SAFA	22.87 ± 0.53 [b]	22.39 ± 1.44 [b]	29.01 ± 0.59 [a]	27.13± 0.54 [a]
MUFA	32.25 ± 0.52 [b]	30.92 ± 1.46 [b]	38.90 ± 0.36 [a]	40.42 ± 0.44 [a]
PUFA	20.87 ± 0.34 [a]	11.81 ± 0.77 [c]	13.19 ± 0.78 [b]	14.53 ± 0.41 [b]
n-3	8.61 ± 0.25 [b]	8.47 ± 0.27 [b]	8.94 ± 0.60 [a]	9.22 ± 0.10 [a]
n-6	12.27 ± 0.12 [a]	3.34 ± 0.49 [d]	4.25 ± 0.18 [c]	5.32 ± 0.43 [b]
n-3:n-6	0.70:1	2.54:1	2.10:1	1.73:1
DHA:EPA	0.18:1	0.19:1	0.23:1	0.18:1
DHA:EPA:ARA	1.27:7.11:1	1.03:5.49:1	1.77:7.70:1	1.16:6.51:1

Means in the same row sharing a common superscript are not significantly different (P>0.05). - = not detectable. SAFA = saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; DHA = docoshexaenoic acids; EPA = eicosapentaenoic acid; ARA = arachidonic acid.

Table 5: Total protein (% dry weight or DW) and amino acid (AA) composition (% of total amino acids) of mixed zooplankton collected from Hanna Dam Lake during study period. Shown are the mean ± SE based on 3 replicates.

	Spring	Summer	Autumn	Winter
Total protein	47.30 ± 3.55 [b]	54.32 ± 1.65 [a]	48.93 ± 1.88 [b]	42.12 ± 3.41 [c]
Essential AA				
Arginine	8.93 ± 0.28 [a]	10.65 ± 0.34 [b]	4.09 ± 0.17 [d]	5.54 ± 0.23 [c]
Cysteine	-	0.25 ± 0.05 [b]	1.44 ± 0.11 [a]	1.46 ± 0.17 [a]
Histidine	1.95 ± 0.17 [a]	1.40 ± 0.05 [b]	1.82 ± 0.17 [ab]	1.77 ± 0.11 [ab]
Isoleucine	0.99 ± 0.23[b]	2.69 ± 0.28 [a]	1.26 ± 0.23[b]	2.72 ± 0.34[a]
Leucine	0.91 ± 0.23 [b]	2.20 ± 0.28 [a]	1.10 ± 0.23 [b]	2.68 ± 0.34 [a]
Lysine	0.90 ± 0.04 [c]	1.23 ± 0.07 [b]	1.39 ± 0.05 [a]	1.41 ± 0.19 [a]
Methionine	2.35 ± 0.23 [a]	2.15 ± 0.23 [b]	1.56 ± 0.17 [b]	4.08 ± 0.28 [b]
Phenylalanine	-	-	2.35 ± 0.11 [a]	-
Valine	2.58 ± 0.23 [b]	2.03 ± 0.17 [bc]	1.75 ± 0.17 [c]	5.34 ± 0.28 [a]
Threonine	6.35 ± 0.28 [a]	4.85 ± 0.23 [b]	6.22 ± 0.28 [a]	7.03 ± 0.34 [a]
Tyrosine	4.57 ± 0.28 [a]	4.71 ± 0.34 [a]	4.07 ± 0.28 [ab]	3.30 ± 0.23 [b]
Total (%)	28.67 ± 0.63 [c]	30.97 ± 0.63 [b]	31.63 ± 0.63 [b]	34.45 ± 0.63 [a]
Total (nmol/mgDW)	94.76 [c]	100.20 [b]	115.21 [a]	74.08 [d]
Non-essential AA				
Aspartic acid	12.3 ± 0.34 [ab]	13.01 ± 0.40 [a]	12.14 ± 0.34 [ab]	11.25 ± 0.28 [b]
Glutamic acid	21.36 ± 0.46 [a]	18.88 ± 0.34 [b]	18.27 ± 0.28 [b]	18.67 ± 0.34[b]
Serine	4.88 ± 0.23 [a]	4.20 ± 0.17 [bc]	3.80 ± 0.11 [c]	4.58 ± 0.23 [ab]
Alanine	16.90 ± 0.40 [a]	15.60 ± 0.34 [b]	14.83 ± 0.28 [b]	12.75 ± 0.23 [c]
Proline	7.27 ± 0.23 [d]	8.64 ± 0.28 [c]	9.61 ± 0.28 [b]	10.98 ± 0.34 [a]
Glycine	8.59 ± 0.23 [b]	8.67 ± 0.28 [b]	9.68 ± 0.34 [a]	7.77 ± 0.17 [b]
Total (%)	71.32 ± 1.84 [a]	69.02 ± 1.84 [a]	68.36 ± 1.84 [a]	67.02 ± 1.84 [a]
Total (nmol/mg DW)	235.74 [b]	223.27 [c]	248.93 [a]	144.08 [d]

Means in the same row sharing a common superscript are not significantly different (P>0.05). - = not detectable.

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Discussion

Water quality and plankton

The high surface water inflow increased water turbidity and reduced Secchi depth due to higher siltation in winter and spring. Based on annual average of Secchi depth, this lake classified as eutrophic lake (Sigee, 2004). In all seasons, the water had suitable DO in epi- and hypolimnion and differences were mostly due to changes in lake surface, volume, and phytoplankton population and microbial decomposition. According to these nutrient concentrations, this lake is classified as eutrophic lake (Auer et al., 1986). Overall, according to water quality criteria Hanna Lake considered as eutrophic lake.

Dinophyceae such as *Ceratium* and *Peridium* are usually present in medium nutrient-rich or mesotrophic lakes (Wetzel, 2001). Of Cyanobacteria, *Microcystis* were dominant throughout the year. Although desmids have a tendency to occur mainly in low nutrient waters (oligotrophic lake), the species of *Cosmarium* and *Staurstrum* are dominated in meso and eutrohic lakes (Bellinger and Sigee, 2010). Overall, according to literature of Reynolds (1990) and Sigee (2004) this lake based on plytoplankton succession, algal bioindicators, and chlorophyll *a* concentration could be considered as mesotrophic-eutrophic during different seasons.

In winter, the cladocerans and copepods were in the lowest population density due to unfavorable reproductive conditions. *D. pulex* was dominant in winter may due better acclimation compared to other cladocerans to extreme fluctuation of water temperatures.

The identified copepod species were all belong to cyclopoids, with highest density observed in autumn (80.38 ind./L) due to its reproduction in wider range of thermal and light conditions (Yusoff et al., 2003) and also lowest NO_3 concentration (Arauzo 2003). Collected copepods were at the different stages of their life, namely; metanauplii, copepodites and adults. The lowest abundance of mesozooplankton in this study was observed in winter may due to highest concentration of NO_3 as reported by Arauzo (2003).

Nutritional values of mesozooplankton

The higher protein in this study was attributed to species composition of mesozooplankton, especially higher density of cladoceran species (Dabrowski and Rusiecki, 1983). For example, protein content in *Daphnia carinata* and *Moina australiensis* were 54.3 and 64.8 % DW, while in some of free-living copepods was 23.0 % DW (Watanabe et al., 1983; Van der Meeren, 2003).

FA composition of mixed freshwater zooplankton obtained in this research provides useful information on nutritive value of mixed zooplankton as food sources. Some PUFA are known to be crucial dietary sources for larval fish and formation of membranes during organogenesis of embryo and larvae. In addition, they have a profound effect on growth, reproduction, survival and larval quality in fishes (Watanabe, 1978; Sargent et al., 1995; Sargent et al., 1999a,

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b; Fountoulaki et al., 2003; Guo et al., 2008). Results showed that the FAs content of mixed zooplankton was changed with variations in the species composition in collected sample. The percentage of SAFA (mainly 16:0) was high in all seasons, which might be related to the feeding of zooplankton from green algae (Chlorophyta and Desmidaceae). This is supported by the fact that myristic acid (14:0) and palmitic acid (16:0) are the major fatty acids in green algae used by zooplankton (Kattner and Krause, 1987; Ahlgren et al. 1992; Brett et al., 2006). Generally, 14:0 and 16:0 are known as precursor of 20:1n-6 and 22:1n-6 FAs, however, the two later FAs were not detected in zooplankton samples of Hanna Dam Lake. This might be due to lack of enzymatic system for chain elongation and desaturation of SAFA in these zooplankton samples (Norsker and StØttrup, 1994; Nanton and Castell, 1999). A high content of MUFA such as 16:1n-7 and 18:1n-9 found in mixed zooplankton samples might be resulted by action of delta-9-desaturase enzyme on SAFA or low content of SAFA in algae used as food by zooplankton (Brett et al., 2006).

As for PUFA, the percentage of total PUFA (mainly 18:2n-6) were greater at spring compared to other seasons. This could be attributed to the composition of zooplankton structure (mostly cladocerans, *Daphnia* spp.) or to the composition of the diet that they feed on. This finding is consistent with the results of Brett et al. (2006) that showed *Daphnia* fed on green algae showed higher amount of 18:2n-6 PUFA. Our results showed that when the higher percentage of zooplankton population was copepod, the higher content of 22:6n-3 (DHA) was present. Contrarily, cladocerans produced lower DHA and EPA content compared to copepods (De Lang and Art, 1999). However, despite the differences among DHA values obtained for different seasons, statistical comparison showed no significant differences. Low contents of n-3 fatty acids, particularly of 18:3n-3 and 20:5n-3 observed in autumn and winter, may be related to feeding mode of these zooplanktons (Claus et al., 1979) as their algal food may differ in fatty acid composition, some having 18:2n-6 and 18:3n-3 and others being rich in EPA and DHA (Bell et al., 1994). In general, DHA level in our zooplankton sample was low (0.73-0.99 %) due to the low ability for elongation and desaturation of short chain n-3 PUFA to long chain n-3 PUFA, diet consumed (Brett et al., 2006), and variation of Hanna zooplankton due to taxonomy and trophic position (Ballantyne et al., 2003; Persson and Vrede, 2006). The n-3 to n-6 ratio in this study ranged 0.70-2.54 in different seasons. This ratio was higher in summer and autumn as mixed zooplankton consisted mainly of copepods (approximately 73 %) with dominant species namely *Metacyclops* sp., *Acanthocyclops* sp., *Microcyclops varicans, Allocyclops* spp., *Diacyclops bicuspidatus, Macrocyclops albidus.* Furthermore, Brett et al. (2006) showed that the ratio of n-3 to n-6 in *Daphnia* is strongly dependent on diet and ranges mainly between 2:1 and 1:1 when fed on

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green algae and blue-green algae respectively. Among water quality parameters, usually water temperature affects the fatty acid composition of zooplankton (Farkas and Herodek, 1964; Norsker and StØttrup, 1994; Nanton and Castell, 1999). In this study, as the temperature decreased, a slight change was observed in amount of PUFA, possibly due to higher proportion of cladocerans, mostly *Daphnia* spp. in our samples. According to Farkas and Herodek (1964), the fatty acid composition of crustacean zooplankton proved to be different and was modified differently by the changes of water temperature.

In this study, the mean EAA and NEAA were 28.7 and 71.3 %; 31.0 and 69.0 %; 31.63 and 68.4 %; 34.5 and 67.0 % of total amino acid in spring, summer, autumn and winter, respectively. Previous study reported by Claybrook (1983) showed that in most crustaceans the NEAA were dominant in AA composition, between 58% and 78%. A change EAA to NEAA ratio could be shown a change in protein metabolism relative to the energetic status of the organism (Graney and Giesy, 1986). In the present study, these ratios were 0.40, 0.45, 0.46 and 0.51 in spring, summer, autumn and winter, respectively. The decreased these ratios were resulted of dietary deficiencies or starvation of mesozooplankton in Hanna Dam Lake.

In this research, alanine and aspartic acid were the next most abundant AAs because of transamination of pyruvate with glutamate (Claybrook, 1983) in crustacean zooplankton.

The relatively great proportion of arginine in current study (4.1-10.7% in total AA pool) can be attributed to the importance of arginine phosphate in crustacean muscle contraction (Onnen and Zebe, 1983). Glycine is normally relatively high in crustacean, and in this study was 7.77 to 9.68 % total AA pool, which may not important for freshwater crustacean due to its specific role in osmotic regulation (Graney and Giesy, 1986). These differences could be attributed to different density of metanauplius, copepodits and adults of copepods in different seasons. The amount of AA in metanauplius was lower compared to copepodit and adult stages of copepods because the surface to volume ratio decreases as the copepod grows. Therefore, the weight of smaller copepod (such as metanauplii) has a larger proportion of exoskeleton, especially chitin which decreases AAs pool. Therefore, in summer AAs pool was higher compared to other seasons in Hanna Dam Lake. In addition, the crustacean zooplankton growth was controlled by periodic molting (Skinner, 1985). Since the frequency of molting is greatest in early instar organisms, with the length of intermolt period increasing with age (Sutcliffe and Caruck, 1981), in summer which the molt cycle interval is short, there is a greater probability of having organisms collected at different stages of molt cycle.

On the other hand, previous study showed that changes in the qualitative

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aspects of an organism diet such as phytoplankton may influence the AA concentration in its tissue and fluids (Brown, 1991). Since the phytoplankton community changed throughout the year, it is possible that the AA of the food may also change seasonally, thus alter the organism AA pool.

Generally, knowledge of seasonal changes in FA and AA composition in zooplankton assemblages in natural conditions will allow the researchers to investigate the changes when described the causes and disturbing agents in stability and health of aquatic ecosystems.

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