Total lipid, fatty acid composition and lipid peroxidation of *Pontogammarus maeoticus* (Crustacea, Amphipoda, Pontogammaridae) in Caspian Sea, Iran

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Abstract. Total lipid, protein, fatty acid contents and lipid peroxidation were investigated in *Pontogammarus maeoticus* in the southern Caspian Sea. The estimated lipid content in males during both seasons (summer and autumn) showed a lower level than females but in autumn lipid level in males was higher than in summer. Protein contents in both genders in autumn were lower than the levels in summer. Nevertheless, such a decrease was more noticeable in females than their male counterparts. Similar fatty acids composition observed in both sexes and they mainly included docosahexaenoic acid, eicosapentaenoic acid, oleic acid, palmitic acid. Polyunsaturated fatty acids (PUFA) levels in both sexes stood higher than other types of fatty acids with different contents in males and females. Females showed greater levels of eicosapentaenoic acid and docosahexaenoic acid than males. The lipid peroxidation rate in males was significantly higher than females ($p \leq 0.05$) whereas it decreased in both genders in autumn. The results indicated that there are sex related variations in *P. maeoticus* in terms of fatty acids and lipid peroxidation. Meanwhile, the high amounts of PUFA are related with decrease in lipid peroxidation levels in this species.

Key Words: lipid composition, live feed, Amphipoda, Caspian Sea.

Introduction. Gammarids are crustacean which play an important role in food chain and purification of organic waste material from environment (Subida et al 2005; Becker et al 2013). These organisms either feed on detritus (wastes left by aquatic herbivores) or provide their energy through predation. They, in turn constitute a major food to various predators including aquatic animals and birds. Thus they convey the energy and the required materials to higher levels of food chain within the environment (Baeza-Rojano 2011; Duffy & Hay 2000). The majority of the economic fish stocks in the Caspian Sea such as beluga, *Acipenser persicus* and herring fishes are heavily dependent upon gammarids. Therefore, gammarids both directly and indirectly play a significant part in provision of food supply and the development of economic fish stocks (Mirzajani 2003).

The nutritional significance of gammarids is further reinforced by their noticeable body reserves of cartenoids, making them an important live food for farmed fish. The inclusion of gammarids as a food ingredient in the diet of cold water farmed fish boosts the nutritional value of fish feed, improved colour and tastes of fish flesh and yields in fish eggs with higher hatching percentages (Conceiçao et al 2010; Vorobyeva & Nikonova 1987).

So far, more than 4300 different amphipod species were identified in various parts of the world living in as diverse environments as oceans, sea shores, deep waters and even fresh water bodies such as streams, rivers, estuaries and bays (Steele & Steele 1969; Subida et al 2005).

Within the Caspian Sea areas, 18 *Gammarus* genera, belonging to 5 gammarids families were identified (Mirzajani & Kiabi 2000; Ghasemian et al 2016; Mirzajani 2004).

Determining the chemical composition and fatty acids within crustacean body is the best means of studying the nutritional biology and unraveling the intricacies of their food requirements (Hyne et al 2009; Kolanowski et al 2007).

The total fatty acid composition in caprellidean genera collected from Strait of Girbraltar were studied (Guerra-Garcia et al 2004; Guerra-Garcia et al 2009). The high amounts of polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid and docosahexaenoic acid were observed among them. Sex related variations in PUFA values were indicated in some species such as *Caprella penanti, Caprella acanthifera* and *Caprella danilevskii*. In addition, sex related and seasonal variations in body chemical composition of *Hyalella pleoacuta* was reported (Dutra et al 2007; Dutra et al 2008).

Pontogammarus maeoticus, a major amphipod species of the Caspian Sea plays an important role in conveying the necessary substances and energy to higher levels of the food chain (Mirzajani 2003). The total lipid and fatty acid composition in amphipods are affected by variations in sex and seasons. Such variations might be dependent upon life cycle stages of the animal as well as other factors such as temperature, salinity and fatty acid contents in their diet (Correia et al 2003; Clarke et al 1985; Hill et al 1992). In addition, the bioenergy reserves and the biochemical parameters in the gammarids might be altered by seasons. Amphipods exhibit a wide range of feeding behavior as benthos feeders, carnivours and herbivours. They, in turn constitute a major food for various aquatic animals (Dauby 2001; Scott et al 2001; Moren et al 2006; Pilgrim & Burt 1993; Normant et al 2004).

Therefore, the present research aims to investigate the fatty acids composition and lipid peroxidation among *P. maeoticus* living along the southern shores of the Caspian Sea with a view to shed more lights on to such compounds within their body and thus gain a better insight of them and their function in the food chain of the area that could pave the way for a more suitable utilization of them in feeding of farmed fish.

Material and Method. The samplings were carried out in summer and autumn 2014. Gammarus were collected from Bandar Anzali shoreline, south west of Caspian Sea (37°28' N and 58°49' E) by hand nets having 1×1 mm mesh size. The netted samples were placed in containers covered with ice and later transferred to the Biology Laboratory of Islamic Azad University, Lahijan, Iran. The physicochemical factors of the designated area were measured and recorded in the field with WTW multi set measurement (Germany). Each pool of whole body of *P. maeoticus* was homogenized on ice in buffer solution (20 mM tris, 1.5 μM phenylmethyl, pH = 7.8) in 1:3 (w/v) ratio range using a hand-held glass-grinder. Total protein was measured in whole body of both genders of *P. maeoticus* according to Bradford (1976) method using Bovine Serum Albumin (BSA) (Sigma-Aldrich) as standard and reading absorbance at 595 nm using a Jenway spectrophotometer (6305 Jenway, UK). Lipid content and fatty acids analysis were performed based on procedure described by Folch et al (1957) involving two phases, lipid extraction and esterification. Chloroform and methanol by a proportion of 1:2 was applied for extracting lipid. In addition, Bromo 3 Foride (BF3) was used for lipid methylation. The identification of fatty acids in the samples were carried out using Phillips Gas cromatograph equipped with column capillary (Gc) BP×70 SGE, (60 m × 0.25 mm) (ID thickness 0.25 μm) and FID detector. The temperature of the detector and injection were set on 26^{o}C and 23^{o}C respectively. The esterified sample (one micro liter) was injected into the gas chromatograph using micro liter syringe. The fatty acid content was determined through comparing the retention time of an unspecified sample with the standard methyl ester fatty acid chromatogram. The fatty acid compounds of the samples were contrasted with standard peak levels. Chromatography Varian Star software (version 6.41) was utilized for sub-peak level measurement and the results presented in μg mg^{-1} body dry weight.

The lipid peroxidation in whole body of *P. maeoticus* was estimated using Ringwood method (Ringwood et al 2003). The procedures involve the formation of malondialdehyde (MDA) in the presence of thiobarbituric acid and reading absorbance at 532 nm. The results are expressed as weight nmol MDA g^{-1} wet^{-1}.

The results were analyzed using SPSS 17 software package. The normalization were first evaluated by Kolmogorov-Smirnov test through the use of Levene test. The total comparison of means was done by one-way ANOVA and and Duncan's Multiple-Range Test. Significance of differences was defined at $p < 0.05$.

Results and Discussion. Seasonally recorded physico-chemical factors are shown in Table 1. Temperature and conductivity were the most variable parameters among the other factors. All other parameters showed stable trend over two seasons.

The results of protein variation in both male and female of *P. maeoticus* are presented in Figure 1. The seasonal variations of protein in males and females show a steady trend. The highest mean protein level recorded was in males (14.47±1.82 mg mL^{-1}) which occurred in summer and the lowest protein level (10.27±1.29 mg mL^{-1}) was in autumn among females. A significant difference was observed in males and females in terms of protein content ($p \leq 0.05$). The mean lipid content in females was higher than males with the lowest mean lipid level occurring in summer 2.83 ±0.91 mg mL^{-1} among males (Figure 1) .

The lipid peroxidation value, mostly measured by MDA showed to be higher in both sexes in summer than in autumn (Figure 2). In addition, it revealed to be significantly greater in males than in females during the two seasons.

Table 1

Physicochemical of water quality in Bandar Anzali at each sampling season

Season	*Temperature (°C)*	*O_2 (mg L^{-1})*	*Conductivity (µS cm^{-1})*	*pH*
Summer	$31±1.24^{a}$	$8.12±0.12^{a}$	$1441±16.22^{a}$	$8.44±0.01^{a}$
Autumn	$15±3.61^{b}$	$8.92±0.22^{a}$	$896±11.06^{b}$	$8.28±0.12^{a}$

Different letters indicate significant differences ($p < 0.05$).

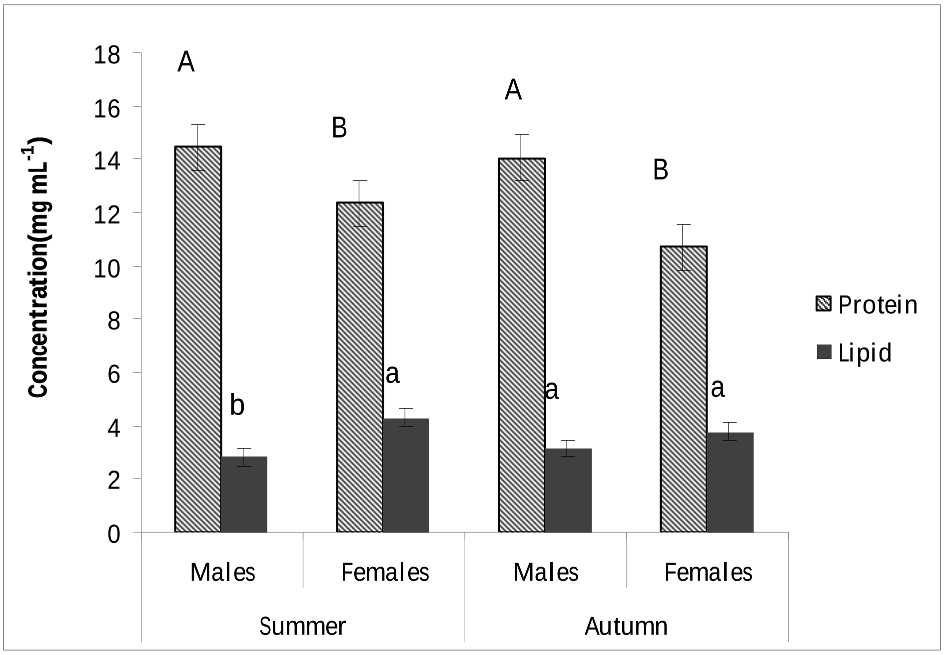

Figure 1. Total lipid and protein levels in the Bandar Anzali at sampling seasons in both genders of *P. maeoticus*. Different letters indicate significant differences ($p < 0.05$).

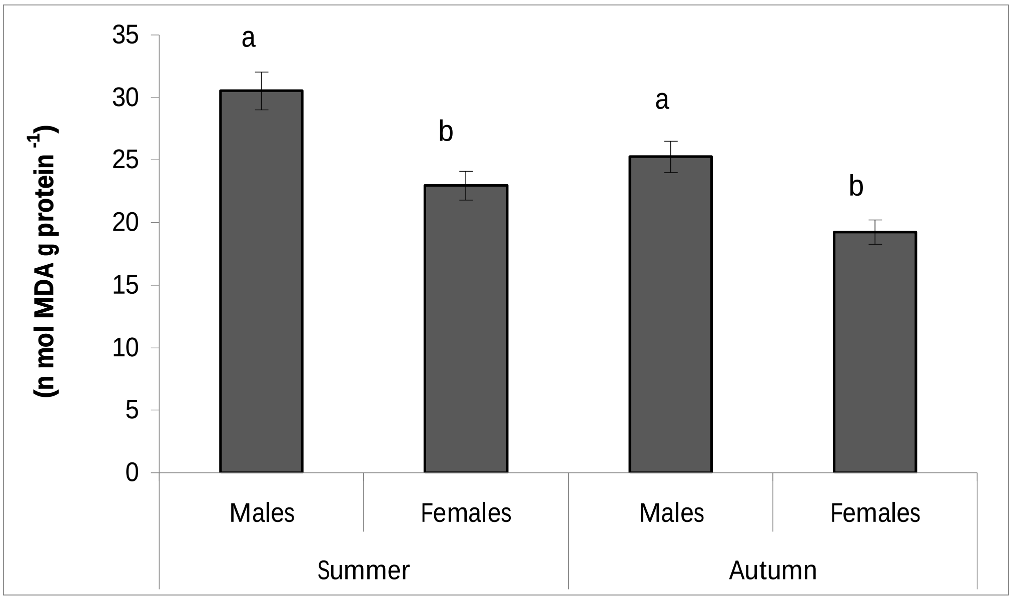

Figure 2. Lipid peroxidation in the Bandar Anzali at sampling seasons in both genders of *P. maeoticus*. Different letters indicate significant differences ($p < 0.05$).

The fatty acid content in the whole *P. maeoticus* body of both males and females during summer and autumn are presented in Table 2.

Table2

Fatty acid compositin for both genders of *Pontogammarus maeoticus* in summer and autumn. The values are expressed as µg mg^{-1} body dry weight. Data are means of three different samples. ND: not detected. Different letters indicate significant differences ($p < 0.05$)

Fatty acids	*Males*		*Females*	
	Summer	*Autumn*	*Summer*	*Autumn*
C14:0	1.05±0.13^{a}	1.13±0.08^{a}	1.04±0.14^{a}	1.17±0.09^{a}
C15:0	0.29±0.06^{a}	0.26±0.05^{a}	0.25±0.08^{a}	0.24±0.03^{a}
C16:0 (Palmitic acid)	2.52±0.35^{a}	2.62±0.40^{a}	2.81±0.55^{a}	2.75±0.51^{a}
C18:0 (Stearic acid)	0.56±0.05^{a}	0.82±0.07^{b}	0.55±0.06^{a}	0.75±0.16^{ab}
C20:0	0.09±0.03^{a}	0.1±0.01^{a}	0.11±0.02^{a}	0.16±0.04^{a}
C16:1n-7 (Palmitoleic acid)	1.56±0.14^{a}	1.86±0.13^{a}	2.20±0.49^{a}	1.93±0.24^{a}
C16:1n-5	ND	ND	ND	ND
C18:1n-9 (Oleic acid)	4.19±0.78^{a}	6.56±0.77^{b}	4.10±0.80^{a}	10.08±1.16^{b}
C18:1n-7 (Vaccenic acid)	1.22±0.12^{a}	1.78±0.21^{b}	1.50±0.37^{a}	1.81±0.21^{a}
C18:1n-5	ND	ND	ND	ND
C20:1n-7	ND	ND	ND	ND
C22:1n-11	0.13±0.02^{a}	0.10±0.02^{a}	0.12±0.03^{a}	0.10±0.02^{a}
C22:1n-9	ND	ND	ND	ND
C18:2n-6	1.41±0.13^{a}	1.60±0.17^{a}	1.30±0.25^{a}	1.47±0.30^{a}
C18:3n-3	0.20±0.02^{a}	0.45±0.05^{b}	0.41±0.17^{a}	0.45±0.06^{a}
C18:4n-3	0.10±0.03^{a}	0.15±0.04^{a}	0.21±0.20^{a}	0.32±0.29^{a}
C20:4n-6 (Arachidonic acid)	3.03±0.25^{a}	3.07±0.35^{a}	1.87±0.30^{a}	2.04±0.37^{a}
C20:5n-3 (Eicosapentaenoic acid, EPA)	3.93±1.01^{a}	11.04±2.50^{b}	4.34±0.67^{a}	13.52±1.21^{b}
C22:5n-6	0.10±0.02^{a}	0.11±0.03^{a}	0.04±0.03^{a}	0.05±0.03^{a}
C22:5n-3	0.81±0.12^{a}	0.73±0.09^{a}	0.16±0.04^{a}	0.27±0.05^{a}
C22:6n-3 (Docosahexaenoic acid, DHA)	2.92±0.45^{a}	5.59±0.95^{b}	1.64±0.35^{a}	7.29±1.55^{b}
Σ Saturated fatty acids (SFA)	4.44±0.07^{a}	5.08±0. 80^{ab}	4.55±0.77^{a}	5.14±0.80^{a}
Σ Monounsaturated fatty acids(MUFA)	7.10±0.86^{a}	10.18±1.31^{b}	7.50±1.11^{a}	13.84±1.45^{b}
Σ Polyunsaturated fatty acids (PUFA)	12.29±1.41^{a}	22.88±1.38^{c}	10.21±1.44^{a}	25.16±2.00^{b}
EPA+DHA	6.85	16.63	5.98	20.81

During summer, fatty acids such as C20:5n-3 and C22:6n-3 (PUFA), C18:1n-9 (MUFA) and C14:0 (SFA) were lower in females than males. The decrease of water temperature in autumn is associated with higher levels of polyunsaturated fatty acids C226n-3, C20:5n-3 and C18:1 among females than males. The total saturated fatty acids (SFA) recorded in males ranged from 5.03±0.04 to 7.02±0.4 µg mg^{-1} body dry weight, whereas the estimated levels for females were 5.22±0.09 and 5.07±0.16 µ mg^{-1} body dry weight. There was also a significant difference between the mean levels of saturated fatty acids during summer and autumn ($p \leq 0.05$). The total saturated fatty acids in females were higher than their male counterparts. A significantly difference in the amount of fatty acids containing monounsaturated fatty acids (MUFA) was detected between the two sexes ($p \leq 0.05$). The MUFA content in males ranged from 6.99±0.24 to 11.45±0.16 µg mg^{-1} body dry weight. Whereas the values for females fluctuated between 7.78±0.24 and 13.97±0.36 µg mg^{-1} body dry weight. The highest fatty acid content as MUFA type was oleic acid (C18:1n-9) which was ranging from 4.12±0.62 to 7.78 ±0.12 µg mg^{-1} body dry weight in males, but estimated values for the females were 3.86±0.56 to 9.71±0.71 µg mg^{-1} body dry weight. The amount of MUFA was affected by seasonal changes in a way that the lowest level was recorded in summer and the highest occurred in autumn in both sexes.

The reproduction period in *P. maeoticus* from Caspian Sea starts in mid spring, reaching its peak in late August. Considering temperature changes during autumn and winter, their spawning in the Caspian Sea occurs during these seasons. However, ovulation process may last longer (Mirzajani 2003, 2004). Thus, the population of females reaches its peak in August. Females gain the highest fat reserves during May to August but the lipid variations within males follows a steady course. In present study the amount of protein in the males were greater than that of in females (Figure 2). This course of changes was relatively stable during the experiment. This could be due to the physiological alterations in both sexes in such a way that proteins within the females might have been used in vetilogenic processes, thus making it smaller than that of in the males. Studies on *Gammaus roeseli* and *Gammaus pulex* revealed a similar downward trend in protein content within the females (Correia et al 2003; Sroda & Cossu-Leguille 2011). Sex related and seasonal variations in energy reserves were investigated in two gammarus species; *Gammarus fossarum* and *G. pulex* (Becker et al 2013). It was found that during winter, these two species have the greatest energy reserves whereas in late summer and early autumn, it declines to its lowest level. The decreased fat during summer might be accounted for by their involvement in reproductive processes (Cook et al 2010). The observed fluctuations of protein content among both sexes in *P. maeoticus* might have been caused by their particular reproductive and physiological condition. The reduced protein level which occurs during autumn and winter could have been due to decreased physiological activities such as reproductive behaviours, and protein in this stage seems to be the main source for ATP synthesis (Becker et al 2016).

Lipid peroxidation rate in both sexes during summer showed to stand higher than autumn and its level among males was significantly higher than females ($p \leq 0.05$). So males has higher capacity to cope with environmental stresses induced by formation of lipid peroxidation among the gammarids (Correia et al 2002). The formation of MDA turned to be more noticeable in males than in females of *G. locusta*. Such a higher MDA level in males could be attributed to male's lower antioxidant capacities as compared to female ones (Correia et al 2003). Maazouzi et al (2007) found that male species of *Dikerogammarus villosus* contained more polyunsaturated fatty acids than the females. These polyunsaturated fatty acids have been recognized as the final goal of reactive oxygen species (ROS). Therefore, the physiological differences between the two sexes can explain the increase of the MDA content in males of *P. maeoticus*.

The observed fatty acid composition in both genders were almost similar and C20:5n, C226n-3, C16:0 accounted for the bulk of fatty acids. The presence of such fatty acids in other species of amphipods such as *G. locusta*, *Caprella* sp. have also been reported (Moore 1976; Cook et al 2010; Rosa & Nunes 2003; Correia et al 2003; Auel et al 2002). In a study on different species of *Caprella*, the fatty acid composition were almost similar across the species belonging to this genus with the exception that certain fatty acids including C18:1n-7, and C20:4n-6 were reported to have been higher in *C. grandim* than other species whereas C:226n-3 was found to be lower in *C. acanthifera* than other species (Garcia et al 2004). In a research by Turan et al (2011) concerning fatty acid composition of brown shrimp, *Crangon crangon*, certain fatty acids including C:182n-6, C18:2n-6, C16:0, C18:1n-9, C20:5n-3, C22:6n-3, C18:2N-6 were of greater prevalence than other fatty acids.

There have also been observable differences within the fatty acids composition of both males and females in *C. penanti, C. acanthifera* and *C. danilevskii* species in that, content of C16:1n-7in females showed to be higher than in males and C20:4n-6 and C20:5n-3 in such female species were found to be less than that of males (Clarke et al 1985). Similar variations in fatty acid content were reported among *G. locusta* (Correia et al 2003). Major fatty acid compounds such as C16:0, C20:5n-3, C22:6n-3 among female of *P. maeoticus* proved to be significantly higher than that of in the male ones ($p \leq 0.05$). Such a difference in fatty acid composition between males and females might be because of varying feeding behavior on the part of female species (due to their feeding on diatom) and their relationship with reproductive behaviours as well as seasonal changes (Correia et al 2003).

Conclusions. In general, the results of this study provide useful information on lipid and protein contents of *P. maeoticus* from Anzali shoreline, Caspian Sea, Iran. Regarding the lipid content, this amphipod show adequate PUFA levels and suitable for use as a natural live feed. The composition of fatty acids in *P. maeoticus* tends to vary with the change in environmental conditions and based on sex-specific qualities but further experimental studies should be done. Enjoying suitable reserves of protein, lipids and polyunsaturated fatty acids, the species can be of high significance in feeding farmed fish.

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