TURKISH JOURNAL OF AQUATIC SCIENCES

RESEARCH ARTICLE/ARAŞTIRMA MAKALESİ ISSN: 2149-9659 E-ISSN: 2528-9462

A PRELIMINARY INVESTIGATION ON THE LIPID CONTENT AND FATTY ACID COMPOSITION OF *Gammarus komareki* (SCHAFERNA 1922), (CRUSTACEA: AMPHIPODA)

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ARTICLE INFO

Received: 03/06/2016

Accepted: 22/08/2016

Published online: 04/12/2016

Odabaşı et al., 31(2): 59-67 (2016)

doi: 10.18864/TJAS201607

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Keywords:

Gammarus komareki
Fatty Acids
Lipid Content
PUFA
Omega-3
Fish Nutrition

Abstract

Beneficial effects of omega-3 (ω-3) fatty acids are consistent of long chain polyunsaturated fatty acids (PUFA) on human health have been well understood issue during the last decade. Freshwater gammarids known as a natural source of PUFA and some of fish especially salmonids (e.g. trout) feeding mainly on these crustacea, thus became rich in these fatty acids. The aim of this work is to reveal of lipid content and fatty acid composition of *Gammarus komareki* that inhabiting in Kırkgözler limnocrene spring Northwestern Anatolia, Çanakkale – Turkey. Total lipid of *G. komareki* was found 4.97% (±0.11). The results showed that the polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were found higher than monounsaturated fatty acids. Total ω-3, ω-6 and ω-9 were 16.63%, 12.3%, and 29.41% respectively. PUFA (30.74%), oleic+elaidic acids (27.84%), palmitic acid (19.63%) and EPA (9.45%) are predominant in *G. komareki*. According to our data, *G. komareki* has almost a higher content of PUFA than the other Gammarids which live in the Europe and Asia. Thus, it might be considered as an alternative source of PUFA and ω-3 fatty acids for local aquaculture nutrition.

INTRODUCTION

The family Gammaridae has a wide distribution mainly centered in Europe and extending to Asia. *Genus Gammarus* Fabricius, 1775, among the Gammaridae is one of the largest genera of Amphipoda, with over 200 species described to date (Vainola et. al., 2008). Gammarids are one of the main foods for trouts and other freshwater fish species (MacNeil et. al., 2000; Graeve et. al., 2001). An important feature of freshwater gammarids is their n-3 PUFA contents (Kolanowski et. al., 2007). Earlier reports on fatty acid composition of benthic invertebrates suggest that dietary PUFAs play a significant role in benthic food webs (Goedkoop et. al., 1998; Gergs et. al., 2014). For example, a variable amount of fatty acids has been estimated in different benthic macroinvertebrates such as trichoptera, and chironomidae (Sushchik et. al., 2003). Because of fish cannot synthesize n-3 PUFA, they are necessary to intake these essential fatty acids by feeding on the invertebrates (MacNeil et. al., 2000). The role of essential fatty acids (PUFA) determining food quality in benthic freshwater invertebrates has been poorly studied. In addition, the relatively higher levels of n-3 PUFA contents have drown feed manufacturers interest as a potential source of valuable feed for cultured fish. For example, one of the fish feed manufacturer has been producing of sun-dried *Gammarus* in China, up to 5000 tons per year (Made-in-china.com, 2015).

According to the studies focused on the Gammaridae in Europe, the freshwater gammarid biomass has been in increasing trend at the last decade due to the migration of so-called invasive species from Caspian and Black Sea Basin to Central and Western Europe via river and artificial channel systems (Grabowski et. al., 2006; Schöll, 2003). The rising trend in gammarid populations biomass bring about the potential use as a source of long chain omega-3 PUFA as well as a natural foodstuff for farmed trout and aquarium fish (Kolanowski et. al., 2007).

Almost all the scientific publications focused on taxonomy, geographic distribution, ecology and life cycle of this genus (i.e. Özbek and Ustaoğlu, 2006; Karaman and Pinkster, 1977; Karaman, 1973; Ustaoğlu et. al., 2004; Duran, 2007; Özbek, 2007; Özbek and Topkara, 2007 etc.), very little study has been issued the compositions of *Gammarus* spp. that taking into account as a foodstuff in the fish feeding industry (Öz, 2009). According to Özbek and Ustaoğlu (2006), 34 taxa of Gammarus among the 48 members of Gammaridae were reported from only continental waters of Turkey.

Although scientific studies have been undertaken on this issue, until now there is no industrial-scale usage of this invertebrate either in Turkey or in Europe but in China. However, hobby purpose usage of those is common among aquarium hobbyists as live or dried food.

On the other hand, according to the earlier data of Karaman & Pinkster (1977), *Gammarus komareki* seems to have pretty much distributional range with a dense population structure (Odabaşı et. al., 2012), specifically in northern regions of Turkey. Thus, this *Gammarus,* native to Turkey, might be considered as a new source for nutritional purpose of aquarium fishes. *Gammarus komareki* is a freshwater Gammarid species with a wide distribution range extending from Bulgaria and northern Greece to southern Russia around Black Sea coasts in the north and to the northern half of Turkey into the north-western part of Iran in the east. This species usually found in running waters or springs and is able to tolerate a relatively high degree of organic pollution (Karaman and Pinkster, 1977). The species belongs to the artificial *Gammarus pulex*-group due to lack of a dorsal carina and dense setation occurring on pereiopods 3, 4 and uropod 3 (Copilaş–Ciocianu, 2013). Although it has a wider range of distribution in Turkey relative to other regions (Karaman and Pinkster, 1977), there is very limited information on its population structure and current ranges.

In this study we aimed to determine the lipid content and fatty acid composition of a *G. komareki* population that inhabiting in a limnocrene spring. This is the first study aimed to determine the lipid content and fatty acid compositions of this species.

MATERIAL AND METHOD

Sampling and Laboratory Studies

Pınarbaşı Kırkgözler spring area where the *Gammarus komareki* population lives located at Çanakkale Province, Northwestern Turkey. *Gammarus komareki* individuals were sampled from its habitat by using D-frame kick net. Besides, some water quality parameters such as pH, dissolved oxygen and temperature were recorded *in*-situ at the time of sampling.

Sampled individuals put into containers that filled by natural habitat's water. Then, living specimens of *Gammarus komareki* transferred to the laboratory while keeping in constant water temperature. In the laboratory, specimens were collected by forceps after sieved and washed with tap water in order to remove foreign material. Then, excess water was eliminated from the sample by lying on a paper towel to a short period of time under laboratory conditions. Sample was weight after a drying process at 70ºC until constant weighed to calculate the moisture ratio.

Total lipid was extracted according to Folch method (Folch et. al., 1957). Five grams of fresh sample were homogenized in a cold mixture of chloroform–methanol (2:1). Solid residue was filtered and washed with a chloroform-methanol mixture. The combined extracts were transferred to a balloon and one quarter of the total volume of distilled water was added. The mixture was shaken fairly and the two phases were allowed to separate. The chloroform phase remained lower was removed, dried anhydrous Na_2SO_4, and then held in rotary evaporator under vacuum at 60 ºC. The lipid phase obtained was covered by nitrogen, weighed and washed out from the evaporation flask using hexane, additionally dried by passing through anhydrous Na_2SO_4 in drying oven, then closed in a small vials under nitrogen. Fatty acids were converted to methyl esters by evaporating hexane with nitrogen flow. The dry lipid fraction was saponified by 0.5 N NaOH and methanol solution, covered with nitrogen, mixed and heated in the water-bath at boiling point for 40 minutes. The saponified material was transmethylated with 14% BF3 in methanol reagent, covered with nitrogen, at boiling point for 3 minutes. After this process, the mixture was cooled and 3 mL hexane was added, covered with nitrogen and shaken strongly for 30 seconds. After separation of phases, the hexane layer was transferred by syringe to the thin flask and additionally dried over anhydrous Na_2SO_4 and decanted to clean vial, covered with nitrogen and closed tightly.2 µL of fatty acid methyl ester was injected in to the chromatograph column by micro syringe under certain conditions with two replicates.

Chromatography

Analysis of fatty acids was performed by using GC-2014 Shimadzu Gas Chromatograph instrument (Japan) equipped with Rtx 2330 silica capillary column of 100 m in length, 0.25 mm ID, df 0.1 mL (Product of Nation). Helium was used the carrier gas at a flow rate of 0.9 ml. A micro syringe injector at 235ºC and flame ionization detector at 250ºC were used. The column temperature program was adjusted as follows: 155ºC initial temperature in 55 minutes, after that increased at 1.5ºC per minute up to final temperature of 210ºC. Sample was analyzed in duplicate. Known standards were compared to identify the peaks (Figure 1). Results were reported as peaks area percentages.

RESULTS AND DISCUSSION

In the present study total lipid and total fatty acid composition of *Gammarus komareki* were analyzed. According to the results, *G. komareki* has a 4.97% (±0.11) of total lipid, 4.55% (±0.28) of total fatty acids in total lipid in wet weight (Table 1). Totally, 30 different fatty acids fractions were detected from total lipid analysis. Both saturated fatty acids (SFAs) and unsaturated (fatty acids (UFAs) were found in the species. Unsaturated fatty acids were higher than the saturated fatty acids in *G. komareki* and the UFAs/SFAs ratio was 2.27%. In *G. komareki*, 11 varieties of fatty acids were the representatives of the SFAs. The palmitic acid (C16:0) was the predominant fatty acid amongst the SFAs with 19.63% (±0.04). Higher amount of oleic acids and palmitoleic acid were found among mono unsaturated fatty acids (MUFA) of the *G. komareki*; 27.84 % and 8.95 % respectively. Among poly unsaturated fatty acids (PUFA), eicosapentaenoic acid C20:5n3 (ω-3) and linoleic acid C18:2n6c (ω-6) were the most abundant fatty acids with 9.45% (±0.02) and 7.55 % (±1.22) respectively. Linolelaidic acid (ω-6) C18:2n6t and α-linolenic acid (ALA, ω-3) C18:3n3 also found in higher ratios in *G. komareki*, 3.19% (±1.06) and 3.63% (±0.05) respectively.

According to the results, seven major fatty acids characterized the fatty acid profile of *G. komareki*, namely the palmitic acid (C16:0) in SFAs, oleic C18:1n9 (n-9), the eicosapentaenoic acid C20:5n3 (ω-3), linoleic acid C18:2n6c (ω-6) and a small amount of α-linolenic acid C18:3n3 (n-3) in PUFA were determined. Those are also the most abundant fatty acids found in studies previously conducted on various species of gammaridae inhabiting in freshwater and marine ecosystems (Kolanowski et. al., 2007; Biandolino and Prato 2006; Clarke et. al., 1985). SFAs and UFAs and its fractions of *G. komareki* given and illustrated in Table 2 and Figure 2.

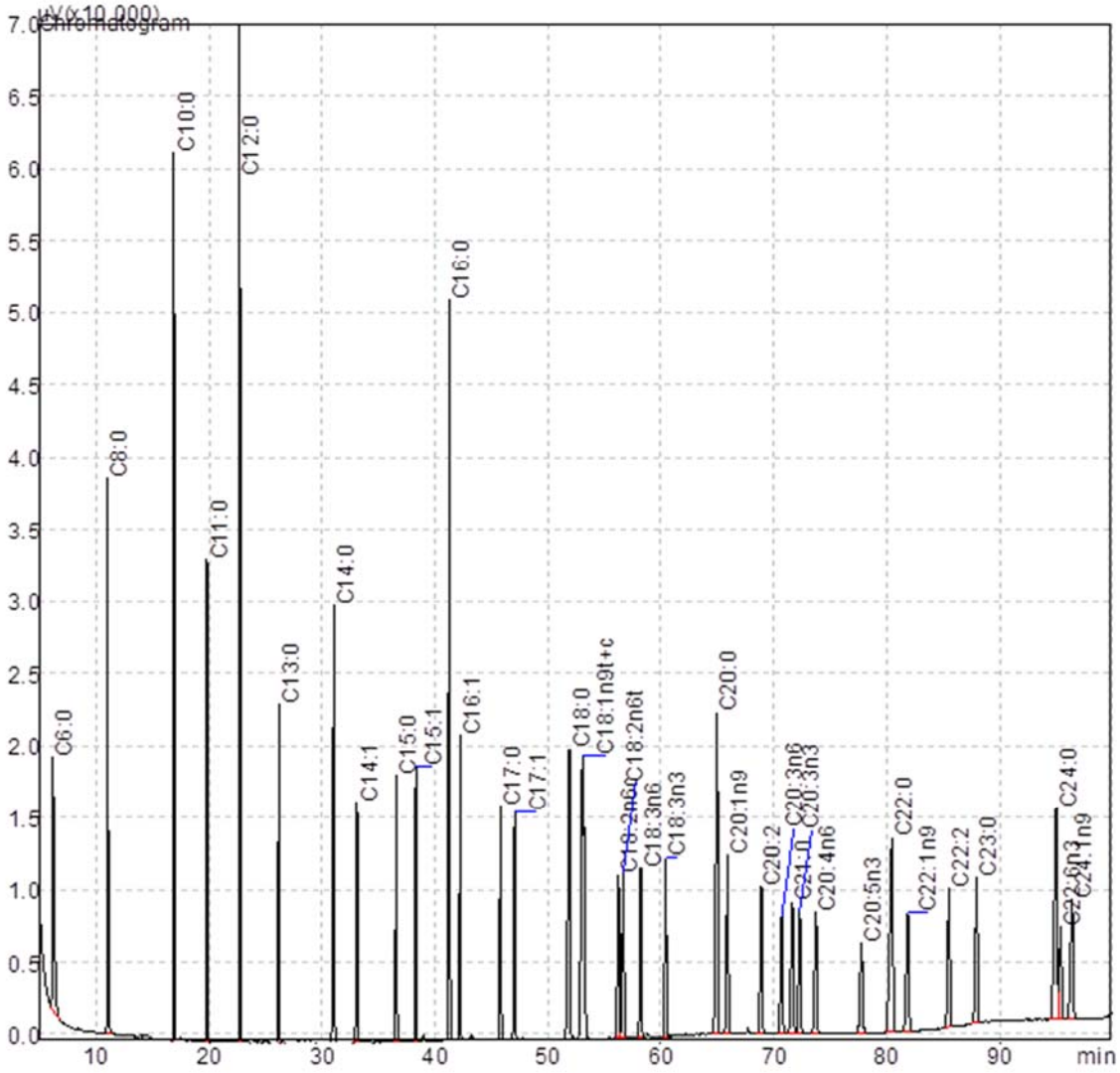

Figure 1. Spectrum of FAME

Table 1. Total Lipid (%) percentages of *G. komareki*.

	Min.- Max.	**Mean ± SD**
Total Lipid (%)	4.89 - 5.04	4.97 ± 0.11
Total Fatty Acids (%) (calculated by GC)	4.35 - 4.75	4.55 ± 0.28
Total Fatty Acids in Total Lipid (%)	89.03 - 94.10	91.57 ± 3.58

Table 2. Fatty acid compositions (% wet weight) of the *Gammarus komareki* in the Kırkgöz Limnocrene Spring

FAME	Min-Max	%mean	SD ±
SFAs			
C11:0	0.00 - 0.05	0.03	0.02
C12:0	0.27 – 0.28	0.28	0.01
C14:0	5.54 - 5.57	5.56	0.02
C15:0	0.42 – 0.43	0.43	0.01
C16:0	19.6 - 19.65	19.63	0.02
C17:0	1.44 – 1.47	1.46	0.02
C18:0	2.47 - 2.70	2.59	0.16
C20:0	0.17 -0.18	0.18	0.01
C22:0	0.12 – 0.22	0.17	0.05
C23:0	0.12 – 0.12	0.12	0.00
C24:0	0.16 – 0.17	0.17	0.01
Total SFAs		**30.62**	
UFAs			
C14:1	0.07 – 0.08	0.08	0.01
C15:1	0.05 – 0.07	0.06	0.02
C16:1	8.93 - 8.96	8.95	0.02
C17:1	0.23 – 0.24	0.24	0.01
C20:2	1.30 – 1.32	1.31	0.01
C22:2	0.36 – 0.63	0.5	0.07
ω-3 Fatty Acids			
C18:3n3 (n-3)	3.59 - 3.66	3.63	0.05
C20:3n3 (n-3)	1.64 - 1.64	1.64	0.00
C20:5n3 (n-3)	9.43 - 9.46	9.45	0.02
C22:6n3 (n-3)	1.91 - 1.91	1.91	0.00
Total ω-3		**16.63**	
ω-6 Fatty Acids			
C18:2n6c (n-6)	6.69 - 8.41	7.55	1.22
C18:2n6t (n-6)	2.44 - 3.94	3.19	1.06
C18:3n6 (n-6)	0.51 - 0.51	0.51	0.00
C20:3n6 (n-6)	0.26 - 0.28	0.27	0.01
C20:4n6 (n-6)	0.74 - 0.81	0.78	0.05
Total ω-6		**12.30**	
ω-9 Fatty Acids			
C18:1n9 (n-9)	27.55 - 28.12	27.84	0.40
C20:1n9 (n-9)	1.20 - 1.21	1.21	0.01
C22:1n9 (n-9)	0.00 - 0.24	0.12	0.17
C24:1n9 (n-9)	0.09 - 0.38	0.24	0.21
Total ω-9		**29.41**	
Total SFAs		**30.62**	
Total UFAs		**69.48**	
UFAs/ SFAs		**2.27**	

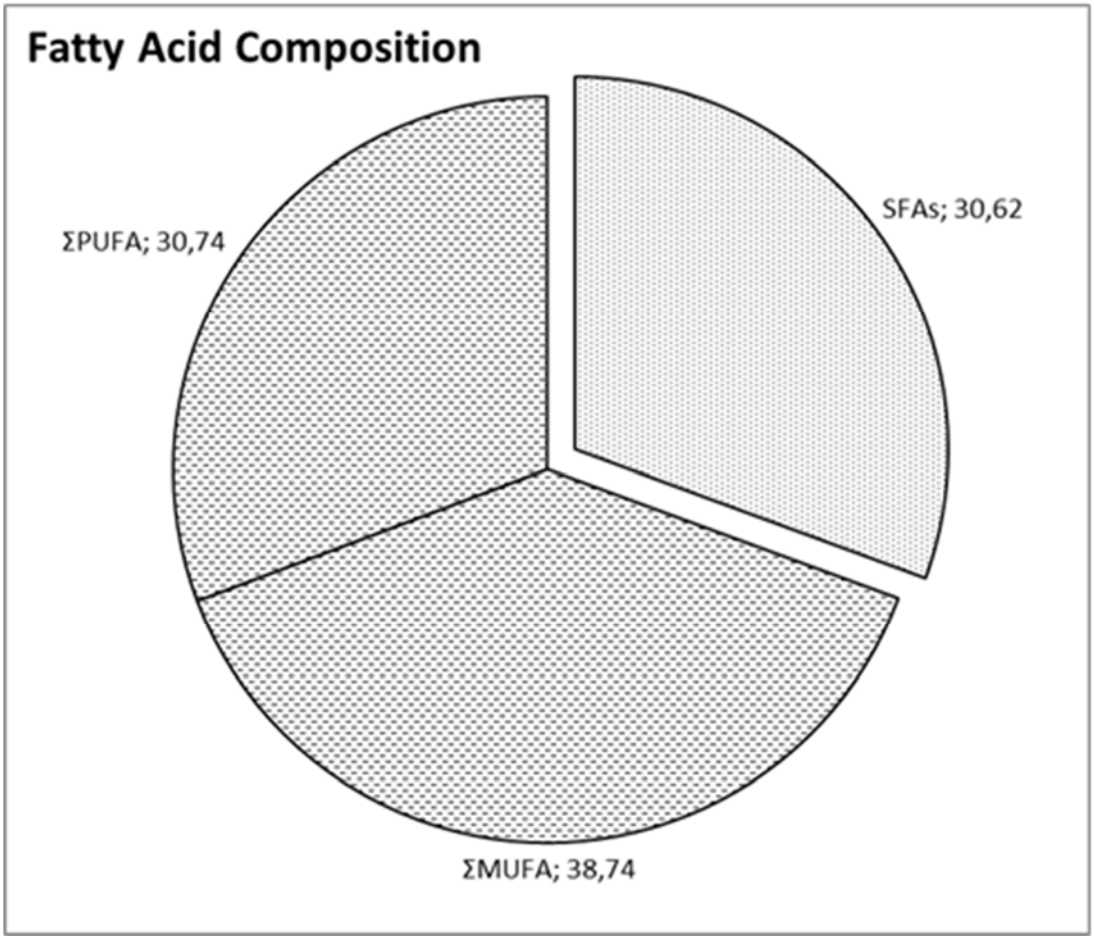

Figure 2. Illustration of fatty acid compositions of *G. komareki*

G. komareki has a higher content of ΣMUFA (38.74%) than those of other freshwaters gammarids i.e. *G. fossarum*, *G. roeseli* (35.5%, 30.03% respectively) (Table 3). *G. komareki* has slightly lower in ΣPUFA ratio with 30.74%, when compared with the other freshwater counterparts such as *Pontogammarus robustoides*, *G. fossarum* and *G. roeseli* which are contain ΣPUFA ranging between 34.3 – 45.8%. But it has contained as much ΣPUFA as in *Dikerogammarus haemobaphes* (32.3%) and *G. pulex* (26.7%) (Arts et. al., 2001). On the other hand, *Eulimnogammarus* (*Pililimnogammarus*) *viridis*, belong to different family of the order amphipoda from Northern Asia (Yenisei River, Russia), content the higher levels of ΣSAT%, ΣMUFA% and ΣPUFA when compared with the species of Gammaridae (Sushchik et. al., 2003). Fatty acid composition of *Gammarus aequicauda*, inhabitant of estuarine habitats (Greze, 1977), showed differences from *G. komareki* within this study. The contents of ΣPUFA and ΣMUFA were significantly higher in *G. komareki* than those of *G. aequicauda*. According to a study by Makhutova et. al. (2003), *G.lacustris* has a lower ΣPUFA and ΣMUFA ratios ranging between 14.71% and 32.58 % when compared with the *G. komareki*. On the other hand, *Dikerogammarus villosus* has a lower content of ΣMUFA (33.61%), while higher ΣPUFA (36.88%) than those of the *G. komareki* (Maazouzi et. al., 2007). According to Öz (2009), the fatty acid composition of *Gammarus pulex pulex* represented similar traits as found in *G. komareki* with the present study and *G. aequicauda*. Apart from, α-linolenic C18:3n3 (n-3) and linoleic C18:2n6c acid fractions of *G. pulex pulex* were significantly higher than those of *G. komareki*.

According to our results, *G. komareki* has an important place among the Gammaridae in terms of the total levels of fatty acids. Considering the higher population density of the *G. komareki* with respect to other congeners, this species could be a useful organism as an alternative source of PUFA for animal nutrition.

Table 3. Saturated, monounsaturated and polyunsaturated fatty acids percentages of various Gammarids

Species / FA Groups	% ΣSFAs	% ΣMUFA	% ΣPUFA	References
Gammarus komareki	30.62	38.74	30.74	Present Study
G. pulex pulex	33.8	39.5	26.7	Kolanowski et. al., 2007
G. lacustris	52.7	32.58	14.71	Makhutova et. al., 2003
G. fossraum	25.2	35.5	39.3	Kolanowski et. al., 2007
G. roeseli	23.9	30.3	45.8	Kolanowski et. al., 2007
G. aequicauda	43.82	33.49	22.74	Biandolino and Prato, 2006
Eulimnogammarus viridis	64.1	55.8	71.6	Sushchik et. al., 2003
Pontogammarus robustoides	28.8	36.9	34.3	Kolanowski et. al., 2007
Dikerogammarus haemobaphes	29.1	38.6	32.3	Kolanowski et. al., 2007
D. villosus	29.51	33.61	36.88	Maazouzi et. al., 2007

Gammarids have already been used as a natural foodstuff for fish nutrition by harvesting of wild populations with high population density (e.g. A China origin unknown *Gammarus* sp. marketed as freeze and sun dried product). Thus, wild populations of many aquatic invertebrates which have an economic value including especially Gammarids, under pressure of over exploitation. As a result of this excessive harvest, some local-scale ecological problems might be expected in near future. In conclusion, we recommend that mass culture of native and in the same time highly efficient species of Gammarids in stable conditions by mimic natural habitats of organism will most likely prevent ecological devastation by over exploitation. On the other hand, recent biotechnological developments might be applied to increase storage capacity of LC PUFA by using suitable organism for gaining much more efficiency from a small amount of living unit.

Acknowledgments

This study was funded by The Scientific and Technological Research Council of Turkey (TÜBİTAK) within the scope of 2209-A: Undergraduate-Graduate Scholarships for Turkish Citizens (Reg. No: 1919B011301374-2014). We are also gratefull to Assoc. Prof. Dr. Umur Önal for his suggestions developing the manuscript.

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