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-RESEARCH ARTICLE-

Seasonal Changes in the Chemical Composition of the Beadlet Anemones (*Actinia equina*) from Mersin Bay, Northeastern Mediterranean coast of Turkey

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Abstract

In this study, the effects of seasonal variation in proximate composition and fatty acid profile as a component of chemical compositions of Mediterranean Sea anemone species (*Actinia equina L.,1758*) living in Mersin Bay were investigated. Chemical composition analysis of anemone samples showed that while the highest levels of protein and water were obtained in winter, the highest lipid and total mineral substance (TMS) levels were obtained in autumn. In terms of fatty acid analysis, during all four seasons the dominant saturated fatty acids (SFA) were palmitic (C16:0) and stearic acids (C18:0), the dominant monounsaturated fatty acids (MUFA) were oleic (C18:1*n*9) and vaccenic acids (C18:1*n*7) and the dominant polyunsaturated fatty acids (PUFA) were linoleic acids (C18:2*n*6), linolenic acid (C18:3*n*3), gamma linolenic acid (C18:3*n*6), arachidonic acid (C20:4*n*6), eicosapentaenoic acid (EPA, 20:5*n*3) and docosahexaenoic acid (DHA, C22:6*n*3) for *A. equina*. The highest values of gamma linolenic acid, EPA and DHA levels were obtained in autumn as 0.44%, 14.83% and 14.10%, respectively.

Keywords:
Actinia equina, Chemical Composition, Lipids, Fatty Acids, Mersin Bay

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Introduction

Sea anemones as a member of Phylum: Cnidaria also known as Coelenterata are common organisms in many benthic marine communities. They are known as omnivorous marine organisms that consume everything they can catch and they can live by adhering to hard substrates such as rocks, corals, other animals or ship bottoms. Sea anemones of the genus *Actinia* are common on many ecosystems and have received much attention from ecologists and many other workers (Perrin *et al.*, 1999). The most common species of this genus, *A. equina* (L., 1758) shows a fairly wide distribution along all the sea shores of the World. One of the most abundant concentrations of *A. equina* is thought to be distributed from the coasts of north Russia to those of South Africa and throughout the northeastern Atlantic. It is very common on shores around the British Isles and Western Europe and extends into the Mediterranean and Black Sea (Perrin *et al.*, 1999; Yanagi *et al.*, 1999; Monteiro *et al.*, 1997; Chomsky *et al.*, 2004a; Stefanov *et al.*, 1992). *A. equina* whose taxonomy is Phylum: Cnidaria, Class: Anthozoa, Subclass: Hexacorallia, Order: Actiniaria, Family: Actiniidae, Genus: *Actinia* is the most common species of sea anemones along coastal areas in Europe and the Mediterranean (Chomsky *et al.*, 2009). Over this very wide geographical distribution *A. equina* is considered to be highly variable in colour pattern, reproductive biology, morphology and habitat choice (Monteiro *et al.*, 1997). Mediterranean Sea anemones (*A. equina L., 1758)* individuals may have different colors mainly green or red (Wirtz *et al.* 2003). Sometimes they may have yellow, green or blue randomly distributed spots. The most common hue is rust-red (Fish & Fish, 1996). It is generally accepted that the red form is most often found in warmer seas, while the green form prefers northern seas (Stefanov *et al.*, 1992).

The anatomy is most easily divided into three parts: the tentacles, the body column and the base. The body column is smooth. The base diameter could be reached to 50 mm which includes the base foot that binds to a solid surface. An *A. equina* specimen has got up to 192 unbanded tentacles which arranged radially in six circles around the opening to the gastrovascular cavity. Bright blue spots, called acrorhagi, are below the tentacles on the outer margin of the column and look like warts. Here include lethal compounds for the purpose of hunting and defending (Fish & Fish, 1996). Although sea anemones *(A. equina L., 1758)* occur in the rocky intertidal zone (Gadelha *et al.*, 2013), it can also live in subtidal areas up to 20 m. (Fish & Fish, 1996; Chomsky *et al.*, 2004b). In Israel, polyps of *A. equina* usually occur at the mid-tide level, in shaded positions under ledges or in caves, but sometimes also are found in unshaded rock pools (Chomsky *et al.*, 2004b).

While some anemones have long tentacles and actively catch benthic prey, *A. equina* has short tentacles and appears to be a suspension feeder. Main source of food for the species *A. equina* is organic detritus as well as bivalve mollusks, insects, and isopods (Chintiroglou & Koukouras, 1992; Kruger & Griffiths, 1997; Kruger & Griffiths, 1998). *A. equina* reproduces both sexually and asexually (Perrin *et al.*, 1999). Although they reproduce sexually by viviparous reproduction, they can also reproduce asexually through parthenogenesis of vegetative growth (Hayward & Ryland, 1995; Chomsky *et al.*, 2009). Although some species of the sea anemones can live longer than 65 years in aquariums, *A. equina* species has a lifespan of approximately 3 years (Fish & Fish, 1996). The sea anemone possesses nematocysts containing toxins for purpose of defence or predation. *A. equina* was newly established to contain a polypeptide toxin (named Ae I) having lethal activity to crabs, besides the well-known cytolytic toxins (equinatoxins) of proteinic nature (Lin *et al.*, 1996).

Chomsky *et al.*, (2004a) have studied genetic variation of *Actinia* populations along the Mediterranean coasts of Israel. On the other hand, it has not been found any research about the proximate composition and fatty acid profile of the species *A. equina* along the Northeastern Mediterranean Coast. Few researches about the chemical compositions of *A. equina* have been

investigated in other different regions than Mediterranean Sea (Ortega & Navarro, 1988; Ortega *et al.*, 1988; Stefanov *et al.*, 1992; Stabili *et al.*, 2015). The chemical composition of the tissue of marine species is defined as the determination of macro and micro level of the components. So, chemical composition of species may change depending on the hunting area and the season. The chemical compositions of marine invertebrate species are of interest for scientists since some of them are known as sources of methylene interrupted PUFA, in particular of the (n-3) and (n-6) series, and also their toxic levels are directly proportional with their protein levels (Bergé & Barnathan, 2005; Yahyavi *et al.*, 2012).

A. equina is a common sea anemone, which produces toxic proteins. Sea anemones possess these toxins, which function as chemical weapons to paralyze prey. These toxic proteins are often used in pharmacology and biotechnology. Equinatoxin which is the one of the toxin produced by anemone is a protein and a member of the family of poreforming toxins, which was isolated from the venom of *A. equina*. Since EqT-II is an eukaryotic pore-forming toxin and is a relatively simple protein, it should be evaluated whether there is a relationship between the toxin level and the protein level of the anemone in subsequent studies.

This is the first scientific study to investigate the effects of seasonal changes in the proximate composition and the fatty acid profile of *A. equina* on the Northeastern Mediterranean coast.

Materials and methods

Sampling

A. equina samples used in this study were collected manually from the sampling zone from winter in 2014 to autumn in 2015 for four seasons in Mersin-Yeşilovacık Bay where they are abundant on rocky shore around (Figure 1, 2). Since the anemone population in the sampling area is widely composed of red species, green anemone individuals that differ in morphological were not included for this study. Therefore 80 red anemone individuals were used in this study totally. Collecting of samples for winter, spring, summer and autumn seasons were realized in 2nd weeks of October 2014, December 2014, April 2015 and July 2015, respectively and 20 individuals were collected for each season. It was taken care of being closed with scales in weight and length of individuals sampled. The weight and width of the individual anemone ranged from 5.4 to 9.7 g and 19 to 29 mm, respectively. At the time of catch, samples were immediately placed in ice. After storage in ice, for no longer than 4 h, they were transferred to the Seafood Processing Technology Laboratory of the Faculty of Fisheries, Çukurova University. Samples were placed in a freezer at -20 °C and stored there 3 months until related analysis.

Figure 1: The specimen of the *Actinia equina* from the Yeşilovacık Bay (photo Dayas)

Figure 2: Map of the sampling location (The marked area is the sampling area)

Proximate analyses

The following chemical constituents were determined on samples of all muscles according to the official methods of analysis of the AOAC (2003): moisture content by oven drying **a ca.** 2-g test sample at 102 ºC to a constant weight (950.46B, see p. 39.1.02); ash content by igniting **a *ca.*** 3–5-g test sample in a muffle furnace at 550ºC until light grey ash results (920.153, see p. 39.1.09); crude protein content by the classical macro-Kjeldahl method (981.10, see p. 39.1.19); and lipid (crude) content by Bligh & Dyer (1959) using chloroform/methanol extraction.

Fatty acid methyl ester analyses (FAME)

Lipid extraction was made according to the Bligh & Dyer (1959) method. Methyl esters were prepared by transmethylation using 2 M KOH in methanol and n-heptane, according to the method described by Ichihara *et al.*, (1996), with minor modification. Extracted oil (10 mg) was dissolved in 2 ml n-heptane, followed by 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2

min at room temperature. After centrifugation at 4000 rpm for 10 min, the *n*-heptane layer was taken for GC analysis.

Gas chromatographic condition

The fatty acid composition was analyzed by the GC Clarus 500 with autosampler (Perkin Elmer, Shelton, CT, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m 0.32 mm, ID 0.25 mm, BP20 0.25 UM; SGE Analitic Science Pty Ltd, Victoria, Australia). The oven temperature was 140 ºC, held for 5 min, raised to 200 ºC at a rate of 4 ºC/min and to 220 ºC at a rate of 1 ºC/min, while the injector and the detector temperature were set at 220 ºC and 280 ºC, respectively. Injection volume was 1 µL and the carrier gas helium was controlled at 16 ps. The split used was 1:100. Fatty acids were identified by comparing the retention times of fatty acid methyl esters with a standard 37-component fatty acid methyl ester mixture (catalog no 18919; Supelco). Triplicate GC analyses were performed and the results were expressed in GC area % as the mean value±standard deviation.

Statistical analysis

For data analysis, each sampling season was subjected to one-way analysis of variance, at the 5% confidence level, using the Duncan multiple range test.

RESULTS

In this study, the effects of seasonal variation in proximate composition (such as protein, lipids, water and TMM) and fatty acid profile as a component of chemical composition of *A. equina* living in Mersin-Yeşilovacık Bay area were investigated.

Proximate composition

The range of annual average variation in protein of sea anemone *A. equina* was determined between 12.27 – 13.07%. The average annual lipid variation range of anemone living in Mersin Bay was determined as between 1.35–1.61% in this study. The lipid content was obtanied low in summer and in winter as 1.36 – 1.35% whereas the level of lipid was found high in spring and in autumn as 1.53 – 1.61%, respectively. Annual average TMS variance range of the anemones living in Mersin Bay is determined to be between 3.11-3.96%. As a result of water analysis performed with *A. equina* samples, the water variation ranges were determined as, in order, 81.38%, 80.37%, 79.42% and 81.67% for spring, summer, autumn and winter (Table 1).

Table 1. Seasonal macro component compositions of anemone (*Actinia equina)*

	Spring $\overline{X} \pm S_X$	**Summer** $\overline{X} \pm S_X$	**Autumn** $\overline{X} \pm S_X$	**Winter** $\overline{X} \pm S_X$
Protein	12.75±5.53[a] (68.48)	12.38±0.80[a] (63.07)	12.27±0.73[a] (59.62)	13.07±0.02[a] (71.30)
Lipid	1.53±0.02[b] (8.22)	1.36±0.09[a] (6.93)	1.61±0.09[b] (7.82)	1.35±0.10[a] (7.37)
Water	81.38±1.86[b]	80.37±1.06[ab]	79.42±1.99[a]	81.67±0.20[b]
TMS	3.11±0.32[a] (16.70)	3.15±0.10[a] (16.05)	3.96±0.34[c] (19.24)	3.62±0.16[b] (19.75)
Total	98.77 (93.40)	97.26 (86.05)	97.26 (86.68)	99.71 (98.42)

$\overline{X} \pm S_X$: Average ± Standard Deviation; Values shown in parenthesis calculated as dry weight basis.
TMS: Total mineral substance; Values in same column with different letters are significantly different ($p<0.05$).

Fatty acid profiles

The seasonal variety of the fatty acid composition of the Mediterranean anemones were determined and SFA/PUA, DHA/EPA, *n6/n3*, *n3/n6* rates were calculated using the fatty acid levels. Main fatty acids of Mediterranean anemones are determined to be SFAs; palmitic acid (C16:0) and stearic acid (C18:0), MUFAs; vaccenic acid (C18:1 *n* 7) and oleic acid (C18:1 *n* 9), PUFAs; linoleic acid (C18:2 *n* 6), gamma linolenic (C18:3 *n* 6), alpha linolenic (C18:3 *n* 3), AA (C20:4 *n* 6), EPA (C20:5 *n* 3) and DHA (C22:6 *n* 3). Annual variation ranges of SFA, MUFA and PUFA levels in Mediterranean anemones are, in order 18.41-21.49%, 9.58-13.34%, 28.96-32.97% (Table 2).

SFA levels are determined to be, in order, 21.49%, 18.41%, 19.63% and 19.37% for spring, summer, autumn and winter. MUFA levels are determined to be, in order, 13.34%, 10.38%, 9.77% and 9.58% for spring, summer, autumn and winter. PUFA levels were determined to be 28.96%, 30.99%, 32.97% and 30.48% for spring, summer, autumn and winter, in order (Table 2).

Palmitic acid levels of Mediterranean anemones reached its highest level in spring with 9.27%, and the levels were, in order 7.29%, 7.97%, and 7.48% in winter, autumn and summer. Highest stearic acid levels were measured in spring. Levels of stearic acid, a saturated fatty acid (SFA), in spring, summer, autumn and winter were determined to be, in order, 7.70%, 7.03%, 7.00%, 7.29% for anemones (Table 2).

Among monounsaturated fatty acids (MUFA), oleic acid (C18:1*n*9) seasonal levels varied between 2.38% and 2.64%, while the vaccenic acid (C18:1*n*7) levels varied between 4.78% - 8.48%. Oleic acid (C18:1*n*9) levels for spring, summer, autumn and winter were determined to be, in order, 2.64%, 2.46%, 2.38%, 2.45% and vaccenic acid (C18:1*n*7) levels for the same were determined to be, in order, 8.48%, 5.68%, 4.78% and 4.98% (Table 2).

Among PUFAs, highest linoleic acid level was measured to occur in spring (2.61%). Seasonally, linoleic acid levels were measured as 2.61%, 1.93%, 1.47% and 1.59% for spring, summer, autumn and winter, respectively. Gamma linolenic acid (C18:3*n*6) and linolenic acid (C18:3*n3*) levels of the Mediterranean anemone for spring, summer, autumn and winter were measured as 0.40%, 0.41%, 0.44%, 0.34% and 0.25%, 0.30%, 0.30%, 0.19%, respectively. Arachidonic acid levels of Mediterranean anemones for spring, summer, autumn and winter were found to be very close, at 0.61%, 0.61%, 0.54%, and 0.53% respectively. Among PUFAs, the level of EPA seasonal levels varied between 12.96 - 14.83% and DHA levels varied between 10.83 -

14.10%. The highest levels of EPA and DHA fatty acids were observed in autumn. EPA fatty acid levels are determined to be, respectively, 12.96%, 13.44%, 14.83%, and 13.63% for spring, summer, autumn and winter. The lowest levels of DHA fatty acid was observed in spring, and the highest level was observed in autumn. DHA fatty acid levels are determined to be, respectively, 10.83%, 13.03%, 14.10%, and 12.76% for spring, summer, autumn and winter (Table 2).

Table 2. Seasonal variations in faty acid compositions of anemone (*Actinia equina)*

Fatty Acids (%)	**Spring** $\overline{X} \pm S_x$	**Summer** $\overline{X} \pm S_x$	**Autumn** $\overline{X} \pm S_x$	**Winter** $\overline{X} \pm S_x$
Saturated fatty acid (SFA)				
C12:0	0.03±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
C14:0	0.84±0.17^{b}	0.55±0.09^{a}	0.69±0.08^{ab}	0.67±0.02^{ab}
C15:0	0.27±0.03^{a}	0.24±0.04^{a}	0.25±0.03^{a}	0.25±0.00^{a}
C16:0	9.27±1.24^{b}	7.29±0.08^{a}	7.97±0.41^{a}	7.48±0.10^{a}
C17:0	2.80±0.18^{a}	2.82±0.15^{ab}	3.20±0.07^{b}	3.16±0.31^{ab}
C18:0	7.70±0.03^{b}	7.03±0.33^{a}	7.00±0.35^{a}	7.29±0.02^{ab}
C20:0	0.58±0.01^{c}	0.48±0.01^{a}	0.52±0.01^{b}	0.52±0.01^{b}
ΣSFA	21.49	18.41	19.63	19.37
Monounsaturated fatty acid (MUFA)				
C14:1	0.07±0.01^{a}	0.07±0.01^{a}	0.06±0.01^{a}	0.08±0.02^{a}
C15:1	0.06±0.00^{a}	0.07±0.01^{b}	0.07±0.00^{b}	0.07±0.00^{b}
C16:1	0.92±0.16^{a}	0.86±0.07^{a}	0.83±0.10^{a}	0.77±0.01^{a}
C17:1	0.64±0.07^{a}	0.74±0.11^{a}	1.17±0.10^{b}	0.73±0.14^{a}
C18:1*n*9	2.64±0.23^{a}	2.46±0.08^{a}	2.38±0.15^{a}	2.45±0.01^{a}
C18:1*n*7	8.48±2.53^{b}	5.68±0.06^{a}	4.78±0.34^{a}	4.98±0.04^{a}
C20:1*n*9	0.53±0.02^{b}	0.50±0.01^{ab}	0.48±0.04^{a}	0.50±0.01^{ab}
ΣMUFA	13.34	10.38	9.77	9.58
Polyunsaturated fatty acid (PUFA)				
C18:2*n*6	2.61±0.65^{b}	1.93±0.02^{a}	1.47±0.10^{a}	1.59±0.03^{a}
C18:3*n*6	0.40±0.02^{b}	0.41±0.01^{bc}	0.44±0.02^{c}	0.34±0.02^{a}
C18:3*n*3	0.25±0.03^{ab}	0.30±0.05^{b}	0.30±0.05^{b}	0.19±0.01^{a}
C20:2 cis	1.30±0.11^{a}	1.27±0.11^{a}	1.29±0.05^{a}	1.44±0.15^{a}
C20:4*n*6	0.61±0.08^{a}	0.61±0.01^{a}	0.54±0.07^{a}	0.53±0.05^{a}
C20:5*n*3	12.96±0.25^{a}	13.44±0.24^{a}	14.83±0.66^{b}	13.63±0.05^{a}
C22:6*n*3	10.83±0.13^{a}	13.03±0.64^{b}	14.10±0.50^{c}	12.76±0.76^{b}
ΣPUFA	28.96	30.99	32.97	30.48
SFA/PUFA	0.74	0.59	0.60	0.64
Σ*n*6	3.62	2.95	2.45	2.46

$\overline{X} \pm S_x$: Average ± Standard Deviation

Values in same column with different letters are significantly different ($p<0.05$).

DISCUSSION

The reason of the low value in protein level of sea anemone was considered that this was due to the high content of water and TMM in species. Stabili *et al.*, (2015) reported that protein level in the mucus of *A. equina* was 24.2 % which was higher level of protein compared to our results. It

was considered that this difference was related to the containing higher levels of protein and also being a complex mixture of proteins and polysaccharides of mucus produced by *A. equina*.

Stabili *et al.,* (2015) claimed that the lipid level of mucus produced by *A. equina* was 0.9 %. It was concluded that the containing low value in lipid level of *A. equina* like other marine invertebrates was characteristic. It was also suggested that being a linear relationship between lipit content and gonad development were related to the time in active period of reproductive metabolism in the autumn and spring seasons. Ortega & Navarro (1988) proclaimed that the lipid levels of *A. equina* were independent on tidal position and they reported that the lipid content in whole tissue of *A. equina* varied seasonally between 1.00 and % 1.75% of the wet weight (5.00 on 8.75 g dry weight basis) irrespective of tidal position. These findings on lipid levels were similar to our study. Stefanov *et al.,* (1992) roperted that the total lipit level of red and green *A. equina* collected to analyse the lipid and fatty acid compositions from Black Sea was 2.2%. Total lipid level determined by researchers was higher than our result. It was considered that this was due to the collecting the samples from different regions and the differences in their size.

High TMS content of Mediterranean anemones compared to vertebrate and invertebrate species of the marine ecosystem can be the result of this species accumulating macro, trace and potentially toxic elements in its habitat. The assessment of the TMS levels of Mediterranean anemones in conjunction with the element composition of seawater and potential food sources is important for determining the source of high TMS levels. Table 1 shows the seasonal changes in the TMS levels of anemones. TMS levels are determined to be, in order, 3.11%, 3.15%, 3.96%, and 3.62% for spring, summer, autumn and winter (Table 1). The levels of other macro components were important in the water levels for each season, and it was determined that the high level of TMM was the chief determiner of the water level. While the water level is highly variable in invertebrates, the anemone water levels are withing the overall ranges of cnidarians.

Highest levels of SFA were found in spring with 21.49% and the lowest level was observed in summer with 18.41%. Highest MUFA level was observed in spring with 13.34% and the lowest in winter with 9.58%. Highest PUFA levels were observed in autumn (32.97%) while the lowest levels (28.96%) were observed in spring (Table 2). In their study, Bergé & Barnathan (2005) point out that representatives of the Coelenterate – cnidarians phylum have peculiar characteristics with regards to fatty acid composition. In an analysis performed on four gorgon species from the genus *Pseudopterogorgia*, it was determined that while PUFAs were dominant, basic PUFA components were, similar to our study; 18:3(*n*-6), 18:4(*n*-3), AA (arachidonic acid), DHA (Docosahexaenoic acid), 24:5(*n*-6) and 24:6(*n*-3) (Bergé & Barnathan, 2005). It was concluded that anemones, known to have fatty acid profiles similar to other marine species found in similar trophic levels in the same marine ecosystem, show some differences with these species with regards to the seasonal variations of fatty acids. Considering the roles of fatty acids in basic metabolism, it can be thought that the seasonal differences of anemone fatty acids compared to other species can be due to seasonal metabolic changes, and their assessment especially alongside breeding metabolism can lead to an explanation.

In a study by Stefanov *et al.,* (1992) it was determined that the fatty acid composition of red *A. equina* collected in the Black Sea was similar to many other marine invertebrates. In said study, it was determined that the level of palmitic acid in *A. equina* was determined to be 13.65%, which is higher than the level in our study. This difference is thought to arise from the place the sampled individuals lived and the differences in size. In a study by Stefanov *et al.*, [1992] stearic acid levels were reported as 7.32% of total lipids by mass. The stearic acid level reported matches our findings.

In a study by Stefanov *et al.,* (1992) ratios of monounsaturated fatty acids oleic acid (C18:1 *n*-9) and vaccenic acid (C18:1 *n*-7) were determined to be, in order, 7.66% and 6.24%. While

vaccenic acid (C18:1 *n*-7) levels are similar to what we found in our study, oleic acid (C18:1 *n*-9) levels show a significant difference. This difference is thought to arise from the differences in the food chain in the Mediterranean and Black Sea ecosystems.

In a study by Stefanov *et al.*, (1992) performed with *A. equina* in Black Sea, the levels of polyunsaturated fatty acids linoleic acid and arachidonic acid were reported as 1.17% and 4.22% respectively. The linoleic acid level reported by the researchers was lower than our findings, and the arachidonic acid level was higher. It is thought that this difference is the result of the study being run in a different ecosystem.

In a study by Stefanov *et al.*, (1992) performed with *A. equina* in Black Sea, the levels of polyunsaturated fatty acids EPA and DHA were reported as 11.02% and 1.77% respectively. While EPA levels reported in this study were similar to our findings, DHA levels appear to be different. The differences in DHA levels are thought to be arising from regional differences.

CONCLUSIONS

It was determined that this was the first study on seasonal changes in the chemical composition of the beadlet anemones (*Actinia equina Linneaus, 1758*) collected from Mersin-Yeşilovacık Bay in North Eastern Mediterranean Sea. This study is intended as a reference study due to the absence of any previously study conducted for this species distributed Mediterranean shore. The determination of seasonal variations in macro components and fatty acid profiles of this species distributed along Mersin coast were the main outputs.

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