

ELEMENT COMPOSITIONS, FATTY ACID PROFILES, AND PROXIMATE COMPOSITIONS OF MARBLED SPINEFOOT (*Siganus rivulatus*, Forsskal, 1775) and DUSKY SPINEFOOT (*Siganus luridus*, Ruppell, 1878)

Abdullah Öksüz*, Ayşe Özyılmaz, Hatice Sevimli

University of Mustafa Kemal, Faculty of Fisheries and Aquaculture, Department of Fishing and Fish Processing Technology, Iskenderun-Turkey

Abstract: Element compositions, fatty acid profiles, lipid levels, and proximate compositions of marbled spinefoot and dusky spinefoot from Northeastern Mediterranean Sea/Turkey were evaluated. Amounts of K and P of both fish were calculated as the predominant elements among the 11 and followed by Na, Mg, and Ca in this study. Apart from the copper, micro elements composition did not change significantly in both species. Saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) levels were the predominant fatty acids in both species respectively. Linoleic (n6) acid and linolenic (n3) acid levels were significantly higher in dusky spine foot than marbled spine foot. The ratio of DHA/EPA in marbled spinefoot and dusky spinefoot were calculated as 3.58 and 1.82, respectively. Lipid content of dusky spine-foot was significantly ($P < 0.05$) higher than that of marbled spine-foot, although moisture contents of both fish were almost the same.

Keywords: *Siganus rivulatus*, *Siganus luridus*, fatty acids, elements, Northeastern Mediterranean Sea

* **Correspondence to:** Abdullah ÖKSÜZ, University of Mustafa Kemal, Faculty of Fisheries and Aquaculture, Department of Fishing and Fish Processing Technology, Iskenderun 31200 (Hatay)-TURKEY

Tel: (+90 326) 6141693 Fax: (+ 90 326) 6141877

E-mail: aoksuz@mku.edu.tr

Özet: Çarpan (*Siganus rivulatus*, Forsskal, 1775) ve Tavşan (*Siganus luridus*, Ruppell, 1878) Balıklarının Element Kompozisyonları, Yağ Asitleri Profilleri ve Besin Bileşenleri

Kuzeydoğu Akdeniz bölgesinde bulunan çarpan ve tavşan balıklarının element kompozisyonları, yağ asitleri profilleri ve besin bileşenleri incelenmiştir. Bu iki balığa ait K ve P seviyeleri incelenen 11 element içerisinde en yüksek miktarlarda olduğu ve bunları sodyum, magnezyum ve kalsiyum elementlerinin takip ettiği gözlenmiştir. Belirlenen mikro elementler içinde yalnız bakır miktarı önemli derecede tavşan balığında, çarpan balığından yüksek bulunmuştur. Her iki türde de en yüksek yağ asitleri doymuş yağ asitleri (SFA) olurken, bunları çoklu doymamış (PUFA) ve tekli doymamış yağ asitleri (MUFA) izlemiştir. Linoleik ve linolenik yağ asitleri tavşan balığında diğer türe göre daha yüksek bulunmuştur. Çarpan ve tavşan balığına ait DHA/EPA oranları sırası ile 3.58 ve 1.82 olarak hesaplanmıştır. Tavşan balığının yağ içeriği çarpan balığından önemli miktarda ($P < 0.05$) yüksek ve bu iki balığa ait nem miktarları ise hemen hemen aynı bulunmuştur.

Anahtar Kelimeler: *Siganus rivulatus*, *Siganus luridus*, yağasitleri, elementler, Kuzeydoğu Akdeniz.

Introduction

Marbled spinefoot, light brown-olive, longitudinal yellow stripes on each side, and dusky spinefoot, body changes from mottled olive-green to dark brown at death (Torcu and Mater, 2000) are two successful lessepsian migrant fish species. These two species draw attention and are considered to be economically important (Torcu and Mater, 2000) fish species due to its delicious taste, considerably low fat content and affordable prize comparing other fish species with very similar visual attributes.

Containing most of the 90 naturally occurring elements, fish are good sources of elements. The greater portion their body consist of carbon, hydrogen, nitrogen, oxygen and sulphur. In addition, six macro elements (calcium, magnesium, phosphorus, sodium, potassium, and chlorine) are present in gram per kilogram quantities. The remaining elements occur in the body in much lower concentrations (mg or per kg) and called microelements (trace elements; iron, zinc, copper, manganese, iodine, cobalt, nickel, molybdenum, chromium, fluorine, etc) (Lall, 1995). Macro elements are required in our diets in amounts greater than 100 mg, and the microelements are required in milligram quantities or less per day (Nabrzycki, 2002).

Fish is generally consumed in almost every part of the world, whether which has a way to the water or not, by humans because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health

in very different ways. This fact has been subjected to many studies and described well enough in too many articles. That is why it is not be detailed these beneficial here.

Though two commercially important lessepsian migrant species, marbled spinefoot and dusky spinefoot belonging to same family in the Northeastern Mediterranean Sea/Turkey, are found in the Mediterranean and consumed along the region, limited information are available about them. The aims of this present study were to search mineral contents, fatty acid profiles, and proximate compositions of marbled spinefoot and dusky spinefoot and also to find out differences between the two species.

Materials and Methods

Materials

Both dusky spinefoot and marbled spinefoot were captured by a local fisherman in the coast of Iskenderun Bay (Northeastern Mediterranean Sea/Turkey). The samples were obtained in March 2008 and analysis was carried out as soon as samples were received.

Sample preparation

After transferring the fish to the laboratory, they were gutted, filleted, and minced. The samples were minced using with a laboratory type blender. Sampling was done in triplicate for each group.

Determination of lipid, moisture, and ash content

EEC recommended oven drying method ISOR 1442 (Commission of European Communities EC, 1979), modified Bligh & Dyer Method (Hanson & Olley, 1963), and A.O.A.C., 35.1.14 (2000) method no 938.08 were used to determine the moisture, crude lipid, and ash content of both fish species, respectively.

Fatty acid methylester (FAME's) and mineral analysis

Separation of FAMES was carried out as described in Oksuz et al. (2009). Fatty acid methyl ester of fish lipid was injected to Hewlett Packard HP 6890 series GC coupled with HP 6890 Mass Selective Detector. Injector was set to automatic mode and injection volume was 2 micro litres on split mode with a split ratio of 1/50. Separation of total fatty acids was achieved in 31 minutes. The identification of individual fatty acids was carried out by comparing those retention time of FAME standard (Supelco 47085U PUFA No: 3) and Supelco 37 component Fame mix (47885-U). Confirmation of FAME was also performed by using a MS data base library (FAMEDBWAX).

Extraction and determination of mineral elements

The wet ashing method was used for digestion of organic matter. This procedure was carried out according to (AOAC Method 975.03) with a minor modification. Fish flesh (2 g) was weighted into a polypropylene screw capped tube and 10 ml of 65% HNO₃ (Merck, Darmstadt, Germany) were added to the sample, followed by the addition of 3 ml of 60 % perchloric acid (Merck, Darmstadt, Germany). Samples were left overnight to complete digestion. Further heating was then carried out in a hot water bath at 90°C for 6 hours. Digests were filtered into a 25 ml volumetric flask, using ash-free filter paper and made up to 25 ml the volume with ultra pure water.

The determination and quantification of mineral elements were done by ICP-AES (Varian Model- Liberty series II). Calibration curves for each of the individual elements were prepared from ICP Multi element stocks (Charleston, SC 29423). The phosphorus standard solution was prepared by dissolving KH₂PO₄ (Merck) in ultra pure water to obtain a 1000-ppm stock phosphorus standard. The standard stock solution was then acidified (100 µl/100 ml) with 65% HNO₃.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's test. Statistical analysis was performed with SPSS 15.0. Significance was established at $P < 0.05$.

Results and Discussion

Lipid, moisture, and ash contents

The lipid, moisture, and ash content of marbled spinefoot and dusky spinefoot are shown in Table 1. The level of lipid content of dusky spinefoot was found significantly ($P < 0.05$) higher than that of marbled spinefoot with the value of 3.31% and 2.37%, respectively. Those values are found higher than those reported for *Siganus rivulatus* in March by Saoud et al., (2008) and Saoud et al., (2007). The amount of moisture content in marbled spinefoot and dusky spinefoot were calculated very similar to the each other (78.1 % and 78.8%, respectively). These similarity were found statistically not significant ($P > 0.05$). Saoud et al., (2008) were reported that level of moisture in dusky spinefoot was found to be 79.11% in March which is slightly higher than that of marbled spinefoot in this study. This differentiation could be the results of different location.

Ash contents of marbled spinefoot and dusky spinefoot were found to be 1.56% and 1.65%, respectively. These values are very close to each other and found statistically not significant ($P > 0.05$).

Spawning season of both marbled spinefoot and dusky spinefoot are supposed to be from April to August in the Mediterranean (Torcu and Mater, 2000). Therefore, both of the fish were supposed to be out of spawning period since they were caught in March.

Table 1. Lipid, moisture, and ash contents of dusky spinefoot and marbled spinefoot

Components (%)	Dusky spinefoot	Marbled spinefoot
Lipid	3.31±0.09 ^a	2.37±0.08 ^b
Moisture	78.8±1.4 ^a	78.0±1.0 ^a
Ash	1.65±0.13 ^a	1.56±0.13 ^a

Means ± SD (n=3) within the same row that have different letter is significantly different ($P < 0.05$).

Fatty acid (FA) composition

The FA composition of dusky spinefoot and marbled spinefoot are shown in Table 2. A total of 20 FA for both fish species were identified in this study. The determination of the fatty acid compositions of dusky spinefoot and marbled spinefoot were performed by GC-MS. The composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acid (PUFA) of dusky spinefoot were found to be 38.62%, 26.15%, and 35.23%, while those of marbled spinefoot were found to be 45.76%, 22.94%, and 31.18%, respectively. The SFA levels in marbled spinefoot were found higher than that of dusky spinefoot whereas the MUFA and PUFA level of marbled spinefoot were present in low levels. The major FA identified in both fish species were palmitic acid (C_{16:0}), oleic acid (C_{18:1 n9}), palmitoleic acid (C_{16:1 n7}), arachidonic acid (C_{20:4 n6}, ARA), docosahexaenoic acid (C_{22:6 n3}, DHA), and eicosapentaenoic acid (C_{20:5 n3}, EPA).

The SFA levels of dusky spinefoot and marbled spinefoot were found to be the highest among all fatty acids. This result may be attributed to the effect of fish digestion. The average level of C_{16:0} in marbled spinefoot (32.64%) was found to be higher than that in dusky spinefoot (28.20%). Similar results were noted in the terms of C_{16:0} for muscle of *Siganus fuscescens*, another spinefoot species, by Osako et al., (2006) and the level of *Siganus luridus* (the same fish species studied in this study) in March by Saoud et al., (2007). The levels of stearic acid (C_{18:0}) and C_{14:0} in both fish species were found to be very close to each other ($P>0.05$). The rest of the SFA components in both fish species were in lower amounts.

The MUFA component of both fish species in this present study were differed from each other. These differentiation were found statistically not significant ($P>0.05$). The level of C_{18:1 n9} and C_{16:1 n7} were the predominant fatty acid in edible part dusky spinefoot and marbled spinefoot. The amount of C_{18:1 n9} in marbled spinefoot was found higher than that of dusky spinefoot with the values of 16.17% and 15.80%, respectively. Osako et al., (2006) reported that the level of C_{18:1 n9} in muscle of *Siganus fuscescens* was found to be 11.7% in March which is the same month that both fish species caught in. The value of C_{18:1 n9} is lower than that of both fish species in this study. However, the level of C_{18:1 n9} in

muscle of *Siganus fuscescens* in May (Osako et al., 2006) was found exactly the same as that of C_{18:1 n9} in dusky spinefoot in March in this study.

The PUFA level of dusky spinefoot was found to be higher than that of marbled spinefoot ($P>0.05$). The DHA in both fish species was calculated as the highest percentages in all PUFA content. Osako et al., (2006) and Saoud et al., (2007) were reported similar result for *Siganus fuscescens* and *Siganus luridus*, respectively. Additionally, the level of EPA in dusky spinefoot was found to be statistically higher than that of marbled spinefoot with the value of 4.34% and 2.36%, respectively ($P<0.05$) in the present study.

Table 2. Fatty acid profiles (%) of dusky spinefoot and marbled spinefoot

Fatty acid	Dusky Spinefoot	Marbled spinefoot
C _{12:0}	0.14±0.06 ^a	1.58±0.11 ^b
C _{14:0}	4.10±0.07 ^a	4.64±0.22 ^b
C _{15:0}	0.30±0.24 ^a	0.38±0.01 ^a
C _{16:0}	28.20±0.32 ^a	32.64±3.30 ^a
C _{18:0}	5.88±0.06 ^a	6.53±0.16 ^b
Total SFA	38.62	45.76
C _{16:1 n7}	10.05±0.07 ^a	6.64±0.27 ^b
C _{18:1 n9}	15.80±0.21 ^a	16.17±0.37 ^a
C _{20:1 n9}	0.30±0.18 ^a	0.13±0.23 ^a
Total MUFA	26.15	22.94
C _{16:4 n1}	0.45±0.05 ^a	0.57±0.38 ^a
C _{18:2 n6}	4.56±0.14 ^a	1.90±0.18 ^b
C _{18:3 n6}	0.74±0.08 ^a	0.00±0.00 ^b
C _{18:3 n3}	1.13±0.12 ^a	0.55±0.08 ^b
C _{20:3 n3}	0.80±0.03 ^a	0.95±0.08 ^b
C _{20:3 n6}	0.90±0.01 ^a	1.12±0.09 ^b
C _{18:4 n3}	0.62±0.06 ^a	0.59±0.01 ^a
C _{20:4 n6}	5.96±0.07 ^a	7.42±0.15 ^b
C _{22:4}	1.22±0.04 ^a	1.76±0.36 ^a
C _{20:5 n3}	4.34±0.03 ^a	2.36±0.26 ^b
C _{22:5 n3}	6.57±0.08 ^a	5.52±0.30 ^b
C _{22:6 n3}	7.94±0.04 ^a	8.45±0.64 ^a
Total PUFA	35.23	31.18
DHA/EPA	1.82	3.58
Total n3	21.41	18.42
Total n6	12.16	10.44
Σ n6 : Σ n3	0.57	0.57

Mean ± SD (n = 3) with different letter in the same row is significantly different.

Level of significance (p < 0.05).

The levels of ARA in both fish species were found surprisingly higher than those of EPA. In addition, the level of ARA was calculated as 5.96% and 7.42% for dusky spinefoot and marbled spinefoot in this study, respectively ($P < 0.05$). Furthermore, the levels of ARA in March for *Siganus fuscescens* and *Siganus luridus* by Osako et al., (2006) and Saoud et al., (2007) were calculated lower than those in the two fish species in this study. However, their results about higher level of ARA are in parallel with our findings. Moreover, having a higher level of ARA than that of EPA could be a distinctive feature for Siganidae family.

The percentage of $\Sigma n3$ PUFA in dusky spinefoot (21.41%) was found higher than that of marbled spinefoot (18.42 %). The value of $\Sigma n3$ PUFA in *Siganus fuscescens* in March (Osako et al., 2006) is similar to that of dusky spinefoot in this present study. The amount of $\Sigma n6$ PUFA in dusky spinefoot (12.16%) was also found higher than that of marbled spinefoot (10.44%). Both of these values are lower than that reported by Osako et al., (2006) for *Siganus fuscescens* in March (16.8%). The ratio of $\Sigma n6 : \Sigma n3$ were calculated as 0.57 for both fish species examined in this research. This value is lower than that reported by Saoud et al., (2007) for *Siganus luridus* in all months they studied.

Element compositions

Element compositions of marbled spinefoot and dusky spinefoot were shown in Table 3. A total of 11 elements was analysed in the edible part of both fish species. As it can be observed from the Table 3, mineral contents of both fish are mostly different from each other, except ratios of Mn (manganese) and Cd (cadmium). Although significant ($P < 0.05$) differences existed between marbled spinefoot and dusky spinefoot only in levels of Cu (copper), no significant ($P > 0.05$) differences were noted in the rest of the element contents studied in this research.

Almost no Cd, a toxic element, was determined in both fish species in this study. This is a good sign for a raw food material. In addition, the average level of Cr (chromium) marbled spinefoot was found to be 0.30 ± 0.10 mg/kg. Even though this value is found a little bit higher than that previously determined for muscle of *Siganus rivulatus* from the coastal waters of EL-max Bay by (Abdallah, 2008), it is still in a very low amounts. On the other hand, the level of Cr dusky

spinefoot was found to be 0.09 ± 0.07 mg/kg which is lower than that of marbled spinefoot in this study and that reported by (Abdallah, 2008) for the same fish species.

The level of Mn calculated in both fish species in this study are the same with each other. This value is lower than in muscle of *Siganus rivulatus* from three different locations (Kress et al., 1999). In addition, the amount of Cu and Zn (zinc) in flesh of marbled spinefoot and dusky spinefoot in this study differed from each other. However, only the levels of Cu between two species were found statistically significant ($P < 0.05$). The levels of Cu and Zn in muscle of marbled spinefoot were found higher than dusky spinefoot in this research. These finding are far below previously determined in both of the same fish species by (Abdallah, 2008). According to Kress et al., (1999) the levels of Cu and Zn in muscle of marbled spinefoot at Haifa Bay were reported similar to those determined in the current study.

The level of Fe (iron) in muscle of marbled spinefoot (8.65 mg/kg) was found lower than dusky spinefoot (9.66 mg/kg) in this research. Compared to Kress et al., (1999) findings, the levels of Fe in muscle of marbled spinefoot at Ardag and Observatory were found higher than our finding. Fe is one of the essential trace elements and should be in daily diet. Fe supplied in the diet must be in the range of 15 mg/day in order to meet daily requirement (Belitz et al., 2004).

The levels of K (potassium) and P (phosphorus) in the edible portion of marbled spinefoot and dusky spinefoot were the most abundant minerals, followed by Na (sodium), Mg (magnesium) and Ca (calcium). The amounts of K and P in the flesh of dusky spinefoot (2880 and 2331 mg/kg, respectively) were found to be higher than those in the flesh of marbled spinefoot (2683 and 2183 mg/kg, respectively) ($P > 0.05$). In addition, fish are a good source of P (Lall, 1995), which is directly involved in energy-producing cellular reactions. On the other hands, K regulates the osmotic pressure within the cell, is involved cell membrane transport, and activation of some enzymes (Belitz et al., 2004).

Although the quantity of Mg in the muscle of dusky spinefoot was found to be higher than that of Mg in muscle of marbled spinefoot with values of 506 and 393 mg/kg, respectively, the dif-

ference between two fish was not statistically significant ($P>0.05$). Even though Mg toxicity is rare, an adequate intake of magnesium has some useful roles in a human body due to the fact that it provides nutrition, regulates enzyme systems, helps to maintain bone health, is required for energy metabolism, and acts as a part of the protein-making machinery in all cells of soft tissues (Whithney & Rolfes, 2008).

Table 3. Mineral compositions (mg/kg, wet weight base) of dusky spinefoot and marbled spinefoot

Minerals	Dusky Spinefoot	Marbled spinefoot
Cd	0.00±0.00 ^a	0.00±0.00 ^a
Cr	0.09±0.07 ^a	0.17±0.16 ^a
Cu	0.08±0.04 ^a	0.30±0.10 ^b
Mn	0.12±0.05 ^a	0.12±0.02 ^a
Zn	5.67±0.56 ^a	6.02±0.61 ^a
Fe	9.66±4.09 ^a	8.65±4.88 ^a
Ca	382.89±131.74 ^a	416.81±71.61 ^a
Mg	506.35±17.17 ^a	392.90±97.09 ^a
Na	687.35±66.52 ^a	729.62±328.50 ^a
P	2331.71±73.85 ^a	2183.61±183.36 ^a
K	2880.14±57.66 ^a	2683.24±370.31 ^a

Mean ± SD (n = 5) with different letter in the same row is significantly different.

Level of significance ($p < 0.05$).

The amount of Ca in marbled spinefoot (417 mg/kg) was found to be higher than that in dusky spinefoot (383 mg/kg) ($P > 0.05$). Ca is an essential mineral which must be added to diet. The amount of recommendation for Ca is (RDA) 800 mg/day. Ca involves in the structure of the muscular system and controls essential process like blood clotting, activity of brain cells and growth (Belitz et al., 2004).

The level of Na in the edible part of marbled spinefoot and dusky spinefoot was found to be 730 and 687 mg/kg, respectively. In addition, no significant ($P < 0.05$) the differences was found in the terms of Na content of both fish species studied in this research.

Conclusions

The levels of ash and moisture were found very similar while those of lipid content differed in both fish species in this current study. However, based on their lipid content both species may be considered as a low fat fish. In addition, the two fish species were rich in unsaturated fatty acid in particular to PUFA such as, DHA, DPA (22:5 n3), ARA, and EPA. Moreover, the levels of P and K in both fish species were found most prevelant macro elements, followed by Na, Mg, and Ca. Based on these results, the two lessepsian migrant fish species seem to be a good source of macro elements, especially P and K. Microelement contents of the *S. luridus* and *S. rivulatus* were almost the same except Cu. The amounts of Cu were found in the range of the provisional tolerable daily intake amounts based on recommended by FAO and WHO for a 60 kg person (FAO/WHO, 1999).

References

- Abdallah, M.A.M., (2008). Trace element levels in some commercially valuable fish species from coastal waters of Mediterranean Sea, Egypt, *Journal of Marine Systems*, **73**: 114-122. doi:[10.1016/j.jmarsys.2007.09.006](https://doi.org/10.1016/j.jmarsys.2007.09.006)
- Belitz, H. D., Grosch, W., Schieberle, P. (2004). *Food chemistry*. ISBN 3-540-40817-75. Springer-Verlag Berlin Heidelberg, Germany.
- Hanson, S.W.F., Olley, J., (1963), Application of the Bligh and Dyer method of lipid extraction to tissue homogenates, *Biochemical Journal*, **89**: 101-102.
- FAO/WHO, 1999. Joint FAO/WHO. 1999. Expert committee on food additives. Summary and conclusions. In: 53rd meeting, Rome, 1–10 June.
- Kress, N., Herut B., Shefer E., Hornung H. (1999). Trace element levels in fish from clean and polluted coastal marine sites in the Mediterranean Sea, Red Sea and North Sea, *Helgoland Marine Research*, **53**: 163-170.
- Lall, S. P. (1995). Macro and Trace Elements in Fish and Shellfish. (Ed.A. Ruiter) In: *Fish and Fishery Products: Composition, Nutritive Properties and Stability*, CAB International, Wallingford, pp. 187-214.

- Nabrzyski, M. (2002). *Mineral Components and functional properties of food components*, Sikorski, Z. E., CRC Press, Florida, chapter. 4.
- Öksüz, A., Özyılmaz, A., Aktas, A., Gercek, G., Motte, J., (2009) A Comparative Study on Proximate, Mineral and Fatty Acid Compositions of Deep Seawater Rose Shrimp (*Parapenaeus longirostris*, Lucas 1846) and golden Shrimp (*Plesionika martia*, A. Milne-Edwards, 1883), *Journal of Animal and Veterinary Advances*, **8**(1): 183-189.
- Osako, K., Saito, H., Kuwahara, K., Okamoto, A. (2006). Year-round high arachidonic acid levels in herbivorous rabbit fish *Siganus fuscescens* tissues *Lipids by AOCS Press*, **41** (5): 473-489.
- Saoud, P. I., Batal, M., Ghanawil, J., Lebbos, N. (2007). Seasonal variation in highly unsaturated fatty acid composition of muscle tissue of two fish endemic to the eastern Mediterranean, *Ecology of Food and Nutrition*, **46**: 77-89. doi:[10.1080/03670240701282456](https://doi.org/10.1080/03670240701282456)
- Saoud, P. I., Batal, M., Ghanawil, J., Lebbos, N. (2008). Seasonal evaluation of nutritional benefits of two fish species in the eastern Mediterranean Sea. *International Journal of Food Science and Technology*, **43**: 538-542. doi:[10.1111/j.1365-2621.2006.01491.x](https://doi.org/10.1111/j.1365-2621.2006.01491.x)
- Torcu, H., Mater S. (2000). Lessepsian fishes spreading along the coasts of the Mediterranean and the Southern Aegean Sea of Turkey, *Turkish Journal of Zoology*, **24**: 139-148.
- Whithney, E., Rolfes, S. R. (2008). *Understanding nutrition* (11th ed.). West Publishing Company, MN (USA), pp. 410.