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Investigation of otolith mass asymmetry of *Squalius* sp. populations sampled from different freshwater sources in Samsun Province (Türkiye)

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Abstract

This research aimed to determine and compare the otolith mass asymmetry (OMA) of Squalius sp. species sampled from ten different freshwater sources in Samsun province. In this context, fish samples obtained from Abdal Stream, Akçay Stream, Engiz Stream, Istavloz Stream, Kaynatma Stream, Mert River, Taşkelik Stream, Terme Stream, Tersakan Stream, Yeşilpınar Stream. The difference between the right and left otoliths were found to be statistically significant in the Istavloz Stream and Tersakan Stream for the asteriscus and in the Abdal Stream for the lapillus ($p<0.05$). OMA (x) and absolute mass asymmetry (|x|) for the asteriscus otolith and lapillus otolith were estimated separately by locality. In all localities, the mean mass asymmetry ($x \pm SE$) values for Sqaulius sp., for the asteriscus otolith and lapillus otolith were 0.00835±0.00280 and -0.00088±0.00246, and the absolute mass asymmetry ($|x| \pm SE$) values were 0.03669±0.00216 and 0.03324±0.00182, respectively. OMA-fish length and absolute mass asymmetry-fish length relationships for asteriscus and lapillus otoliths were determined using the linear relationship equation. According to the results of this study, there were no statistically significant differences among the localities in terms of asteriscus OMA/absolute mass asymmetry and lapillus OMA/absolute mass asymmetry (Kruskal Wallis Test, $p>0.05$). This is the first study in which OMA and absolute otolith mass asymmetry of Squalius sp. living in the freshwaters of Samsun province were investigated, and their relationships with fish length were evaluated.

Keywords: *Leuciscidae, Squalius, otolith, mass asymmetry, Samsun*

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Samsun (Türkiye) ilindeki farklı tatlı su kaynaklarından örneklenen *Squalius* sp. populasyonlarında otolit kütle asimetrisinin incelenmesi

Öz

Bu araştırmada Samsun ilindeki on farklı tatlısu kaynağından örneklenen Squalius sp. türünün otolit kütle asimetrisinin (OMA) belirlenmesi ve lokasyonlar arasında karşılaştırılması hedeflenmiştir. Bu kapsamda balık örnekleri Abdal Çayı, Akçay, Engiz Çayı, Istavloz Çayı, Kaynatma Deresi, Mert Irmağı, Taşkelik Deresi, Terme Çayı, Tersakan Çayı, Yeşilpınar Deresi'nden yakalanmıştır. Sağ ve sol otolitler arasındaki fark asteriskus için Istavloz Çayı ve Tersakan Çayı'nda; lapillus için Abdal Çayı'nda istatistiksel olarak önemli bulunmuştur (p<0,05). Her bir lokalitede, OMA (x) ve mutlak kütle asimetrisi (|x|) asteriskus ve lapillus için ayrı ayrı değerlendirilmiştir. Asteriskus ve lapillus için ortalama kütle asimetrisi (x±SE) değerleri sırasıyla 0,00835±0,00280, -0,00088±0,00246; mutlak kütle asimetrisi (|x|±SE) ise 0,03669±0,00216 ve 0,03324±0,00182 olarak hesaplanmıştır. Asteriskus otolit ve lapillus otolit için OMA-balık boyu ve mutlak kütle asimetrisi-balık boyu ilişkisi linear ilişki denklemi kullanılarak belirlenmiştir. Bu çalışmanın sonuçlarına göre, asteriskus OMA/mutlak otolit kütle asimetrisi ve lapillus OMA/mutlak otolit kütle asimetrisi açısından lokaliteler arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (Kruskal Wallis Testi, p>0,05). Bu çalışma, Samsun ili tatlısularında yaşayan Squalius sp. türünün OMA ve mutlak otolit kütle asimetrisinin incelendiği ve balık boyu ile ilişkilerinin ele alındığı ilk çalışmadır.

Anahtar kelimeler: *Leuciscidae, Squalius, otolit, kütle asimetrisi, Samsun*

1. Introduction

Otoliths are calcified structures in the inner ear of the teleost fish and play a significant role in both balance and hearing senses [1-4]. The otolith is markedly species-specific and continues to grow throughout the life of the fish [5, 6]. Otoliths identify fish species but also have the ability to record life histories and reflect environmental pressures at different stages of their lives [7-9]. There are three pairs of otoliths, the lapillus, sagitta, and asteriscus, which connect to sensory cells (macula) in the utriculus, sacculus, and lagena, respectively [2, 4, 10]. In most Teleost fishes, the sagitta is the largest otolith; however, in Cypriniformes, Characiformes, Gymnotiformes, and Siluriformes, the asteriscus and/or lapillus are larger than the sagitta [5, 11]. Otoliths generally have a bilaterally symmetrical structure in fish, but their weights are different between the left and right otoliths, and this is called otolith mass asymmetry [12]. Otolith asymmetry occurs when the mass and shape of the paired otoliths of an individual are not equal [13-15]. This variability in weight asymmetry is presumed to reveal the growth anomalies caused by genetic or environmental pressures on fish [16]. Although it is known that there is a significant impairment in vestibular and auditory functions, it is observed that the precise quantitative, morphological, and physiological basis of otolith asymmetry is still unclear [17]. In recent years, teleost fish have been playing an important role in the study of asymmetry in otolith mass because they are a very suitable biological model to assess the physiological role of otolith mass [17, 18]. When the literature was examined, it was found that there were studies on otolith mass asymmetry (OMA), absolute mass asymmetry and fish size-OMA/ absolute mass asymmetry relationships in many different marine and freshwater fish [14, 17, 19-30].

Squalius (Bonaparte, 1837) genus, commonly known as chub, belongs to the family Leuciscidae [31]. The genus *Squalius* is one of the most widely distributed inland fishes in the freshwaters of Türkiye and comprises a number of medium-sized fishes widely [32-34]. Due to their omnivorous feeding behavior, they have the ability to consume a wide variety of plant and animal-based foods. There are many different studies such as feeding, age and growth, genetics, and systematics of *Squalius* [35- 41]. The number of studies on otolith mass and shape asymmetries conducted in Turkey is quite low [23- 25, 28- 30]. There is no study on the OMA of *Squalius* in Türkiye in the current literature. In this study, OMA and absolute mass asymmetry were investigated in ten different *Squalius* sp. populations living in Samsun province. In addition, both OMA and absolute otolith mass asymmetry relationships of total length were examined.

2. Materials and methods

Squalius sp. samples were obtained from ten different locations identified in some freshwater sources in the Samsun province of Türkiye. 40 samples were captured in each location, including Abdal Stream, Akçay Stream, Engiz Stream, Istavloz Stream, Kaynatma Stream, Mert River, Taşkelik Stream, Terme Stream, Tersakan Stream, Yeşilpınar Stream between May and November 2022. The electroshock device (SAMUS 725MP) was used for capturing the samples. Fish samples obtained from fieldwork were transported to the Ichthyology Research Laboratory of Ondokuz Mayıs University in containers with ice belonging to the respective locality, and foreign materials on them were removed by washing.

The total lengths of each fish were measured (±0.1 cm). The otolith pairs of the utricular (asteriscus) and lagenar (lapillus) were removed, distinguishing between right and left. Undamaged pairs of otoliths were cleaned and stored in eppendorf tubes. Left and right otoliths were weighted (±0.0001 g). Otolith mass asymmetry (x) was calculated from equation 1, where M_R and M_L are the otolith masses of the right and left otoliths, respectively. M is the mean mass of M_R and M_L. The otolith mass asymmetry value is 0 (zero) when the right and left otolith masses are equal ($M_R = M_L$). A value of zero indicates the absence of otolith mass asymmetry. However, this value can vary between -2 and +2. When the value of X is negative, the left otolith mass is greater than the right otolith mass, and when it is positive, the opposite is true [27].

$$x = (M_R - M_L)/M \tag{1}$$

In this study, the equation 2 was used to establish the linear relationships between (x)-total length and absolute otolith mass asymmetry (|x|)-total length. In this formula, ‘‘b’’ is the coefficient characterizing the growth rate of the otolith, TL is the total length of the fish, and ‘‘a’’ is a constant (intercept) for the given species [18].

$$X = b.TL + a \tag{2}$$

When comparing the otolith weight values of the right and left otoliths, a normality test was applied to the data. If the data were normally distributed, paired sample t test was used for comparisons, and if the data was not normally distributed, the non-parametric Wilcoxon test was applied. The Kruskal-Wallis test, which is used when data are not normally distributed, was used to compare whether there were differences in otolith mass asymmetry and absolute mass asymmetry between localities. SPSS 21.0 software package and the Microsoft Excel packages were used for all statistical analyses.

3. Results

In the study, a total of 400 fish individuals belonging to *Squalius* sp. living in the freshwater resources of Samsun province were caught. The total length of the fish ranged from between 6.5-36.80 cm (13.62±0.17). Right asteriscus, left asteriscus, right lapillus, and left lapillus otolith weight ranged from 0.0005-0.0183 g, 0.0005-0.0186 g, 0.0006-0.0280 g and 0.0006-0.0281 g, respectively. Descriptive statistics of total length, asteriscus otolith, and lapillus otolith weights of *Squalius* sp. according to localities were given in Table 1. There were statistically significant differences between localities in terms of total length (Kruskal-Wallis test, $P<0.05$).

Table 1. Descriptive statistics of total length, asteriscus otolith, and lapillus otolith weights of *Squalius* sp. according to localities (TL: Total length, N: Number of samples, Min.: Minumum; Max.: Maximum; SE: Standard error, SD: Standard deviation)

Locality	**Variable**	**N**	**Min.**	**Max.**	**Mean**	**±SE**	**±SD**
Abdal Stream	TL (cm)	40	9.30	36.80	15.66	0.80	5.04
	Right asteriscus (g)	40	0.0012	0.0183	0.0034	0.0004	0.0028
	Left asteriscus (g)	40	0.0011	0.0186	0.0034	0.0005	0.0029
	Right lapillus (g)	40	0.0018	0.0280	0.0050	0.0007	0.0044
	Left lapillus (g)	40	0.0019	0.0281	0.0051	0.0007	0.0044
Akçay Stream	TL (cm)	40	10.20	22.30	13.38	0.39	2.47
	Right asteriscus (g)	40	0.0014	0.0054	0.0026	0.0002	0.0010
	Left asteriscus (g)	40	0.0014	0.0053	0.0025	0.0002	0.0010
	Right lapillus (g)	40	0.0017	0.0069	0.0035	0.0002	0.0013
	Left lapillus (g)	40	0.0017	0.0069	0.0035	0.0002	0.0013
Engiz Stream	TL (cm)	40	6.50	23.20	13.40	0.58	3.67
	Right asteriscus (g)	40	0.0005	0.0073	0.0027	0.0002	0.0015
	Left asteriscus (g)	40	0.0005	0.0073	0.0027	0.0002	0.0015
	Right lapillus (g)	40	0.0006	0.0079	0.0032	0.0003	0.0017
	Left lapillus (g)	40	0.0006	0.0081	0.0033	0.0003	0.0017
Istavloz Stream	TL (cm)	40	7.50	20.00	13.27	0.53	3.38
	Right asteriscus (g)	40	0.0006	0.0050	0.0020	0.0001	0.0009
	Left asteriscus (g)	40	0.0006	0.0050	0.0019	0.0001	0.0009
	Right lapillus (g)	40	0.0007	0.0069	0.0026	0.0002	0.0013
	Left lapillus (g)	40	0.0007	0.0066	0.0026	0.0002	0.0013
Kaynatma Stream	TL (cm)	40	8.60	21.50	13.08	0.40	2.53
	Right asteriscus (g)	40	0.0009	0.0055	0.0020	0.0001	0.0009
	Left asteriscus (g)	40	0.0009	0.0054	0.0020	0.0001	0.0008
	Right lapillus (g)	40	0.0010	0.0070	0.0026	0.0002	0.0012
	Left lapillus (g)	40	0.0011	0.0072	0.0026	0.0002	0.0012

Table 1. (Continued)

Mert River	TL (cm)	40	10.10	21.00	13.63	0.45	2.85
	Right asteriscus (g)	40	0.0010	0.0045	0.0021	0.0001	0.0009
	Left asteriscus (g)	40	0.0010	0.0047	0.0021	0.0001	0.0009
	Right lapillus (g)	40	0.0014	0.0067	0.0029	0.0002	0.0014
	Left lapillus (g)	40	0.0014	0.0067	0.0029	0.0002	0.0013
Taşkelik Stream	TL (cm)	40	6.60	27.70	13.20	0.64	4.04
	Right asteriscus (g)	40	0.0005	0.0120	0.0025	0.0003	0.0019
	Left asteriscus (g)	40	0.0005	0.0120	0.0025	0.0003	0.0019
	Right lapillus (g)	40	0.0007	0.0165	0.0034	0.0004	0.0026
	Left lapillus (g)	40	0.0007	0.0166	0.0034	0.0004	0.0026
Terme Stream	TL (cm)	40	6.60	17.60	11.30	0.35	2.21
	Right asteriscus (g)	40	0.0005	0.0041	0.0019	0.0001	0.0007
	Left asteriscus (g)	40	0.0005	0.0040	0.0019	0.0001	0.0007
	Right lapillus (g)	40	0.0006	0.0059	0.0029	0.0002	0.0011
	Left lapillus (g)	40	0.0007	0.0062	0.0029	0.0002	0.0011
Tersakan Stream	TL (cm)	40	13.10	19.60	15.75	0.20	1.29
	Right asteriscus (g)	40	0.0017	0.0040	0.0026	0.0001	0.0005
	Left asteriscus (g)	40	0.0017	0.0040	0.0026	0.0001	0.0005
	Right lapillus (g)	40	0.0019	0.0056	0.0034	0.0001	0.0007
	Left lapillus (g)	40	0.0019	0.0056	0.0034	0.0001	0.0008
Yeşilpınar Stream	TL (cm)	40	10.30	17.60	13.55	0.25	1.56
	Right asteriscus (g)	40	0.0016	0.0040	0.0027	0.0001	0.0005
	Left asteriscus (g)	40	0.0016	0.0042	0.0027	0.0001	0.0005
	Right lapillus (g)	40	0.0020	0.0059	0.0038	0.0001	0.0009
	Left lapillus (g)	40	0.0021	0.0057	0.0038	0.0001	0.0009

As a result of the analyses, there were no statistically significant differences between the right and left otoliths of asteriscus in terms of otolith weight for Abdal Stream (Wilcoxon test, P= 0.928), Akçay Stream (Wilcoxon test, P= 0.212), Engiz Stream (Wilcoxon test, P= 0.086), Kaynatma Stream (Wilcoxon test, P= 0.413), Mert River (Wilcoxon test, P= 0.741), Taşkelik Stream (Wilcoxon test, P= 0.317), Terme Stream (Wilcoxon test, P= 0.699), and Yeşilpınar Stream (Paired samples t-test, P= 0.897). However, significant differences were found between samples from the Istavloz Stream (Wilcoxon test, P= 0.018) and the Tersakan Stream (Paired samples t-test, P= 0.028).

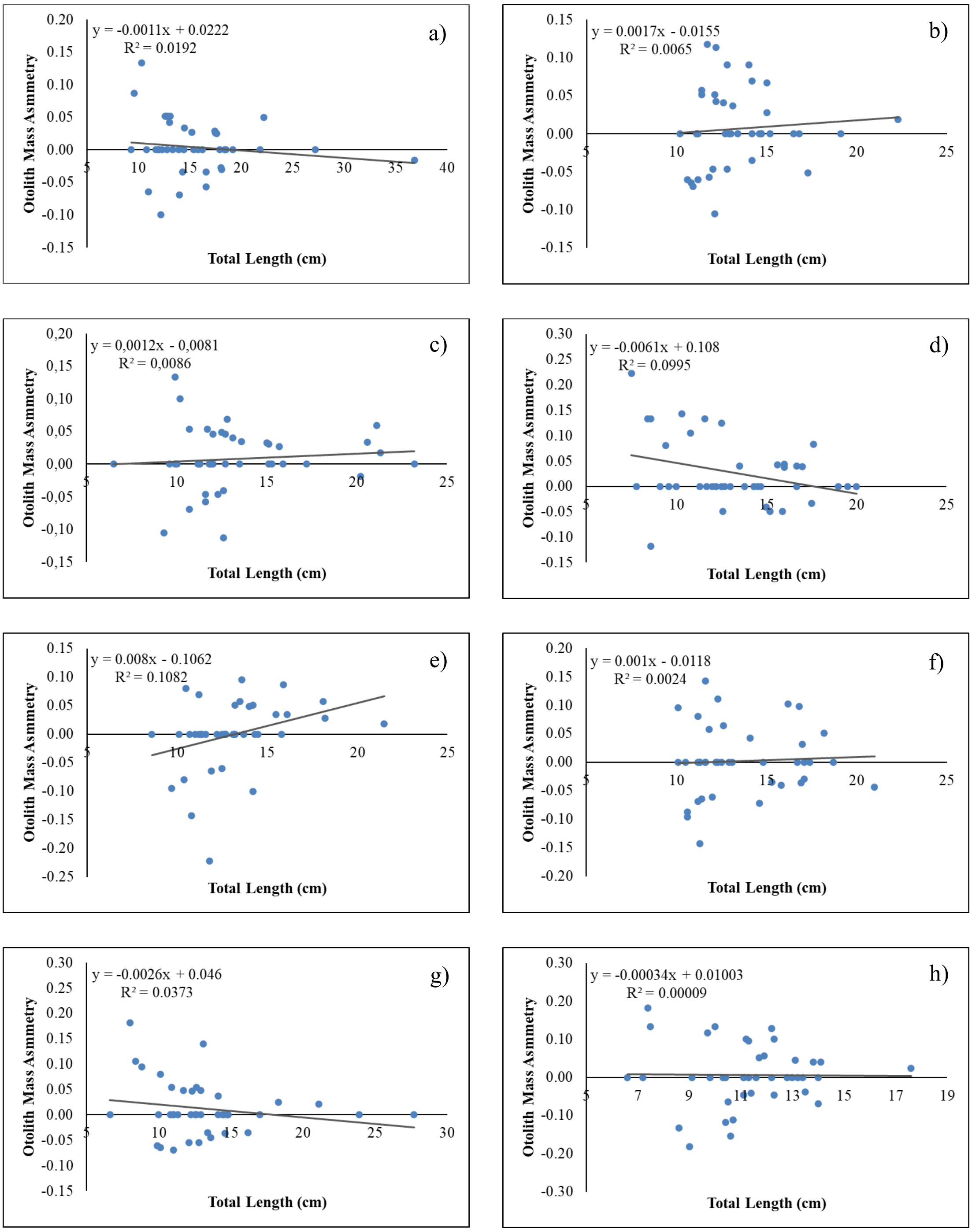
For lapillus in terms of otolith weight, there were no statistically significant differences between the right and left otoliths of samples from Akçay Stream (Wilcoxon test, P= 0.811), Engiz Stream (Wilcoxon test, P= 0.169), Kaynatma Stream (Wilcoxon test, P= 0.736), Mert River (Wilcoxon test, P= 0.664), Taşkelik Stream (Wilcoxon test, P= 0.944), Terme Stream (Paired samples t-test, P= 1.000), Tersakan Stream (Wilcoxon test, P= 0.856), and Yeşilpınar Stream (Paired samples t-test, P= 0.856). However, significant differences were found between samples from Abdal Stream (Wilcoxon test, P= 0.016).

Descriptive statistics of asteriscus otolith mass/absolute mass asymmetry and lapillus otolith mass/absolute mass asymmetry according to localities were given in Table 2. The asteriscus OMA and absolute otolith mass asymmetry were calculated within the range of $-0.22222 \leq x \leq +0.22222$ and $0.00000 \leq |x| \leq +0.22222$ for *Squalius* sp. populations, respectively. The lapillus OMA and absolute otolith mass asymmetry were calculated within the range of $-0.15385 \leq x \leq +0.22222$ and $0.00000 \leq |x| \leq +0.22222$ for *Squalius* sp. populations, respectively. The mean OMA values for asteriscus and lapillus otoliths were calculated as 0.00835 and -0.00088, respectively.

Table 2. Descriptive statistics of asteriscus otolith mass/absolute mass asymmetry and lapillus otolith mass/absolute mass asymmetry according to localities (x: Otolith mass asymmetry, |x|: Absolute otolith mass asymmetry)

Locality	Otolith type	Variable	Min.	Max.	Mean	±SE	±SD
Abdal Stream	Asteriscus	x	-0.10000	0.13333	0.00436	0.00656	0.04148
		|x|	0.00000	0.13333	0.02596	0.00512	0.03239
	Lapillus	x	-0.07407	0.10000	-0.01106	0.00538	0.03402
		|x|	0.00000	0.10000	0.02584	0.00387	0.02446
Akçay Stream	Asteriscus	x	-0.10526	0.11765	0.00693	0.00815	0.05156
		|x|	0.00000	0.11765	0.03676	0.00575	0.03636
	Lapillus	x	-0.10526	0.10526	0.00264	0.00625	0.03950
		|x|	0.00000	0.10526	0.02530	0.00477	0.03018
Engiz Stream	Asteriscus	x	-0.11321	0.13333	0.00829	0.00765	0.04836
		|x|	0.00000	0.13333	0.03316	0.00566	0.03579
	Lapillus	x	-0.11765	0.11111	-0.01748	0.00811	0.05126
		|x|	0.00000	0.11765	0.04245	0.00523	0.03306
Istavloz Stream	Asteriscus	x	-0.11765	0.22222	0.02667	0.01039	0.06574
		|x|	0.00000	0.22222	0.04355	0.00881	0.05573
	Lapillus	x	-0.09524	0.22222	0.01143	0.00914	0.05782
		|x|	0.00000	0.22222	0.03488	0.00746	0.04721
Kaynatma Stream	Asteriscus	x	-0.22222	0.09524	-0.00138	0.00975	0.06168
		|x|	0.00000	0.22222	0.03689	0.00776	0.04909
	Lapillus	x	-0.11321	0.08451	-0.00404	0.00696	0.04402
		|x|	0.00000	0.11321	0.03142	0.00485	0.03069
Mert River	Asteriscus	x	-0.14286	0.14286	0.00251	0.00956	0.06047
		|x|	0.00000	0.14286	0.04132	0.00691	0.04373
	Lapillus	x	-0.11111	0.10526	-0.00144	0.00705	0.04457
		|x|	0.00000	0.11111	0.03003	0.00516	0.03262
Taşkelik Stream	Asteriscus	x	-0.06897	0.18182	0.01212	0.00850	0.05375
		|x|	0.00000	0.18182	0.03478	0.00671	0.04241
	Lapillus	x	-0.07407	0.19355	0.00718	0.00715	0.04524
		|x|	0.00000	0.19355	0.02826	0.00566	0.03578
Terme Stream	Asteriscus	x	-0.18182	0.18182	0.00614	0.01279	0.08089
		|x|	0.00000	0.18182	0.05628	0.00913	0.05773
	Lapillus	x	-0.15385	0.20000	0.00606	0.01216	0.07689
		|x|	0.00000	0.20000	0.05404	0.00859	0.05436
Tersakan Stream	Asteriscus	x	-0.07143	0.11321	0.01531	0.00671	0.04245
		|x|	0.00000	0.11321	0.02875	0.00547	0.03457
	Lapillus	x	-0.09231	0.10526	0.00312	0.00652	0.04122
		|x|	0.00000	0.10526	0.02781	0.00479	0.03027
Yeşilpınar Stream	Asteriscus	x	-0.07407	0.08696	0.00251	0.00674	0.04261
		|x|	0.00000	0.08696	0.02940	0.00484	0.03058
	Lapillus	x	-0.12658	0.08955	-0.00525	0.00678	0.04289
		|x|	0.00000	0.12658	0.03235	0.00446	0.02819

There were no significant differences between localities in terms of asteriscus otolith mass asymmetry (x) (Kruskal-Wallis test, $P>0.05$) and absolute otolith mass asymmetry (|x|) (Kruskal-Wallis test, $P>0.05$) for *Squalius*. The relationship between OMA-total length was found to be significant ($p<0.05$) in Istavloz Stream and Kaynatma Stream locations. However, there was no relationship between OMA and total length in all other locations (p>0.05). When the relationship between otolith absolute mass asymmetry and total length was evaluated, a significant relationship was found in Istavloz Stream, Terme Stream, and Tersakan Stream locations. In contrast, no significant relationship was found in all other locations. Linear relationship equations for asteriscus otolith mass asymmetry-total length in Figure 1 and asteriscus otolith absolute mass asymmetry- total length in Figure 2 for all localities were shown. Regression coefficient values of asteriscus x- total length and |x|-total length relationships were found $0.0001 \leq r^2 \leq 0.1082$; $0.0009 \leq r^2 \leq 0.1703$, respectively.



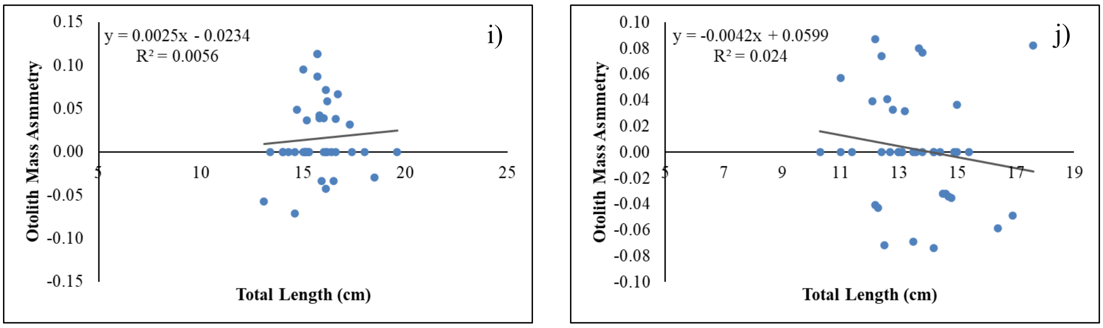
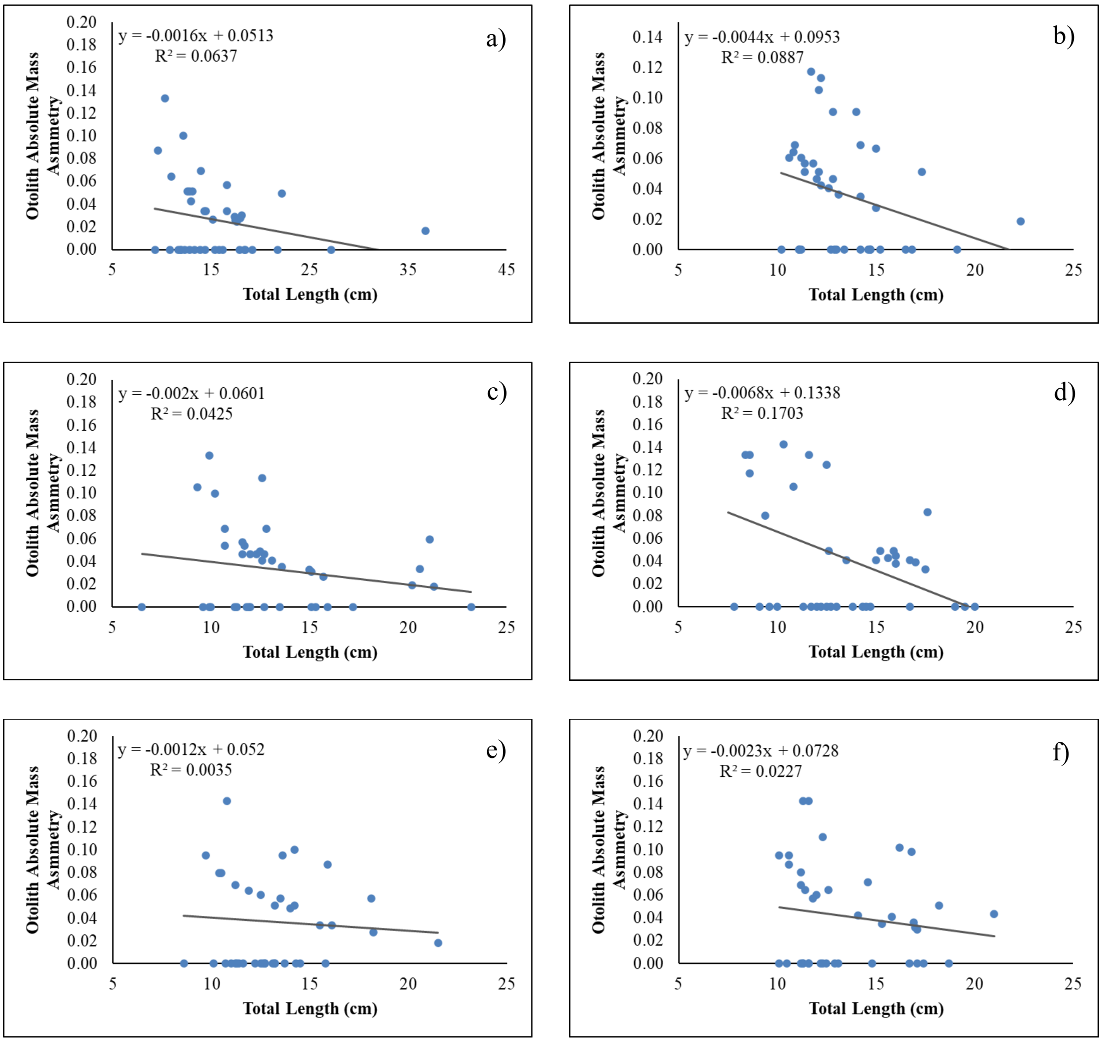


Figure 1. The relationships between asteriscus otolith mass asymmetry and total length in *Squalius* sp. a) Abdal Stream, b) Akçay Stream, c) Engiz Stream, d) Istavloz Stream, e) Kaynatma Stream, f) Mert River, g) Taşkelik Stream, h) Terme Stream, i) Tersakan Stream, j) Yeşilpınar Stream.



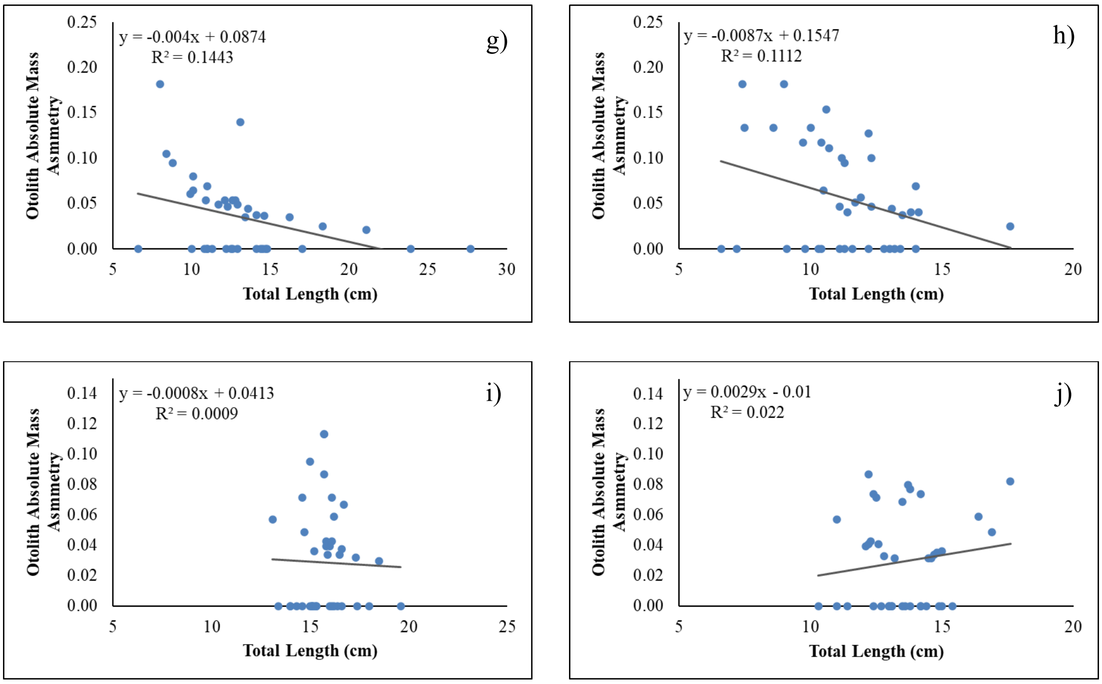


Figure 2. The relationships between asteriscus otolith absolute mass and total length in *Squalius* sp. a) Abdal Stream, b) Akçay Stream, c) Engiz Stream, d) Istavloz Stream, e) Kaynatma Stream, f) Mert River, g) Taşkelik Stream, h) Terme Stream, i) Tersakan Stream, j) Yeşilpınar Stream.

There were no significant differences between localities in terms of lapillus otolith mass asymmetry (x) (Kruskal-Wallis test, P>0.05) and absolute otolith mass asymmetry (|x|) (Kruskal-Wallis test, P>0.05) for *Squalius*. The relationship between OMA- total length was found to be significant (p<0.05) in the Akçay Stream, but there was no relationship between otolith mass asymmetry and total length in all other locations (p>0.05). When the relationship between otolith absolute mass asymmetry and total length was evaluated, a significant relationship was found in the Abdal Stream, Akçay Stream, Taşkelik Stream, and Terme Stream locations, while no significant relationship was found in all other locations.

Linear relationship equations for lapillus otolith mass asymmetry- total length in Figure 3 and lapillus otolith absolute mass asymmetry- total length in Figure 4 for all localities were shown. Regression coefficient values of lapillus x- total length and |x|-total length relationships were found $0.0014 \leq r^2 \leq 0.0502$; $0.0000 \leq r^2 \leq 0.2391$.

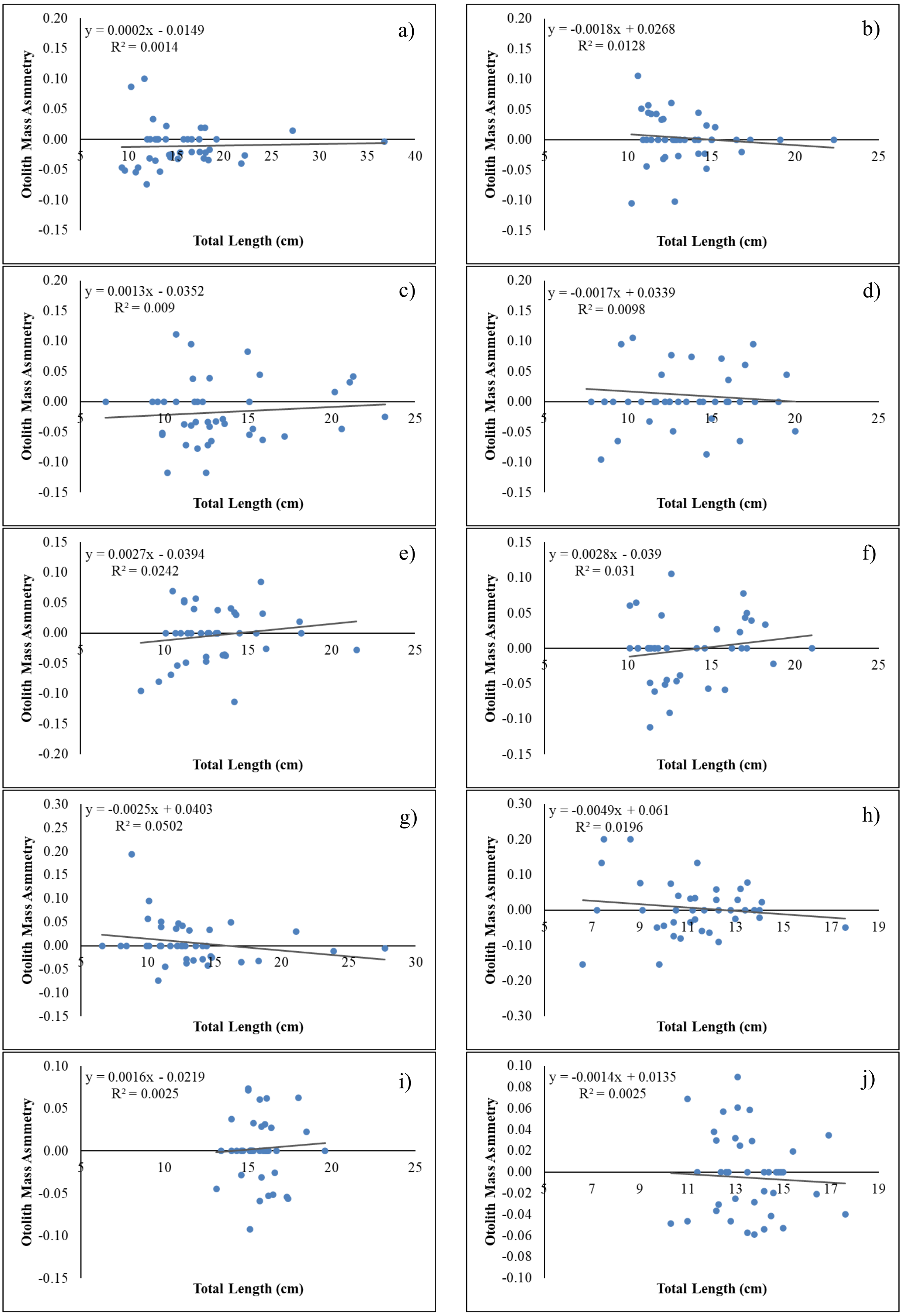


Figure 3. The relationships between lapillus otolith mass asymmetry and total length in *Squalius* sp. a) Abdal Stream, b) Akçay Stream, c) Engiz Stream, d) Istavloz Stream, e) Kaynatma Stream, f) Mert River, g) Taşkelik Stream, h) Terme Stream, i) Tersakan Stream, j) Yeşilpınar Stream.

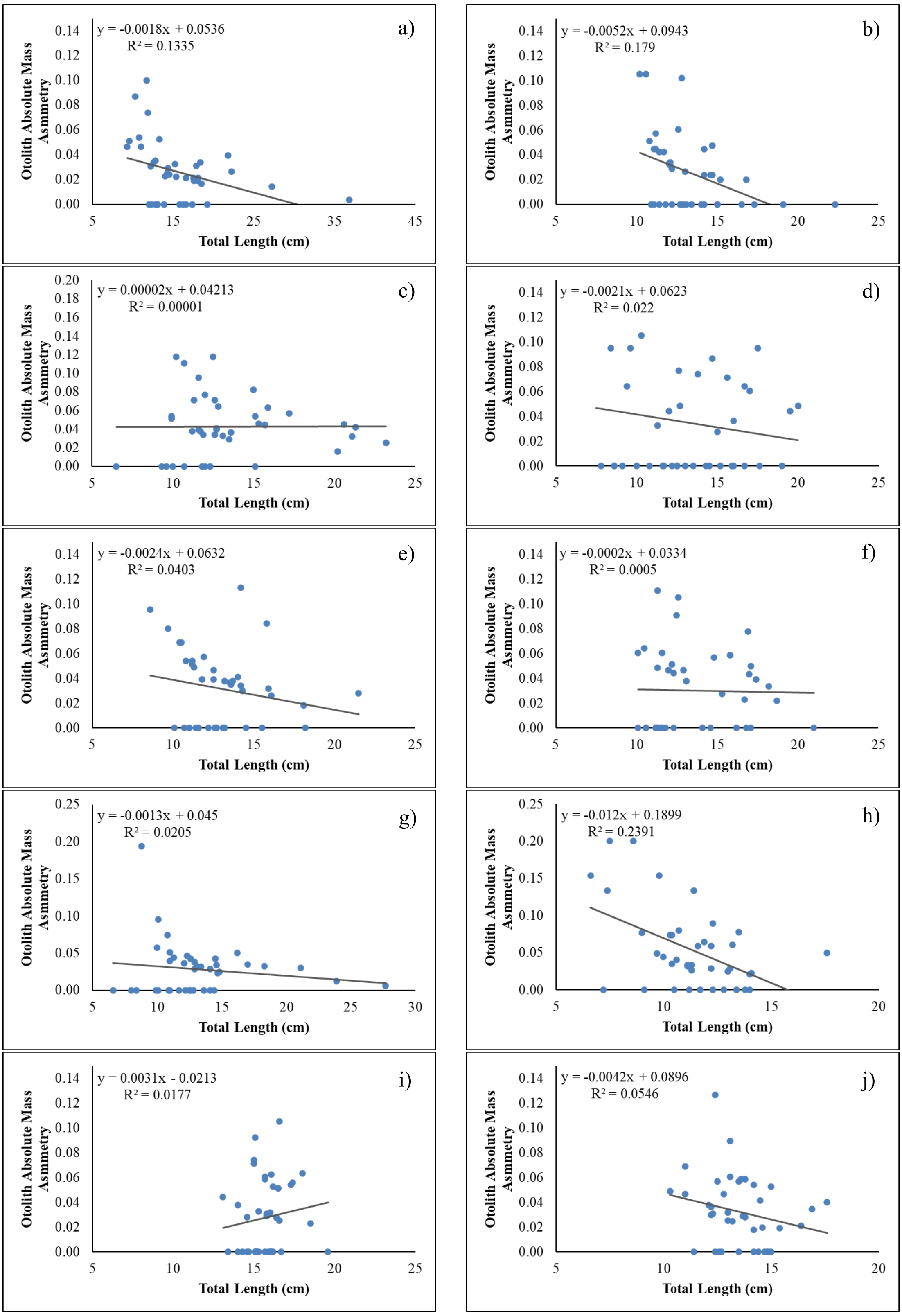


Figure 4. The relationships between lapillus otolith absolute mass and total length in *Squalius* sp. a) Abdal Stream, b) Akçay Stream, c) Engiz Stream, d) Istavloz Stream, e) Kaynatma Stream, f) Mert River, g) Taşkelik Stream, h) Terme Stream, i) Tersakan Stream, j) Yeşilpınar Stream.

4. Discussion

Otolith mass asymmetry in fish can vary during individual fish growth [24]. In fisheries research, there are many studies on OMA were found to be in the range of $-0.2<x<+0.2$ for marine and freshwater species [13, 17, 23, 24, 27, 29, 42- 44]. According to the results of the present study, this value for asteriscus otolith and lapillus otolith was ranged from $-0.22222 \leq x \leq +0.22222$ and $-0.15385 \leq x \leq +0.22222$ for all localities, respectly. The results of our study demonstrated that both asteriscus otolith and lapillus otolith fell within that range across all populations. Asymmetry in the utricular and lagenar otolith organs of symmetric fish species is usually low [45]. Furthermore, Lychakov & Rebane [13] stated that, in principle, only fish with large otoliths and $|x|>0.2$ may experience difficulty in sound processing due to the improper and inconsistent movement of the two otoliths on both sides of the fish's head. In this study, $|x|$ is very low for asteriscus otolith and lapillus otolith. When the literature was examined, no study was found in Turkiye, where OMA and absolute otolith mass asymmetry of *Squalius* were examined. The average otolith mass asymmetry for asteriscus otolith in different fish species living in Turkish freshwater was calculated as -0.00803 ± 0.00642 for *Capeota banarescui* [25]; 0.0685 ± 0.0194 for *Barbus tauricus* [24]. In the current literature, there are studies on OMA and absolute otolith mass asymmetry of different fish species [17, 23, 24, 27-30, 42-44, 46, 47].

Grønkjær [48] and Izzo et al. [49] have reported in their studies that otoliths are directly influenced by the environmental conditions of the habitats in which the fish reside. Both natural variations in environmental factors and anthropogenic influences have significant effects on the development of otoliths [50]. Asymmetry in otolith weight can impact and diminish the hearing functionality of a fish's ear [13]. In theory, a discrepancy in the movement of the right and left otoliths only occurs if the absolute value of X exceeds 0.2, and this condition can alter the acoustic functionality of a fish [13, 17]. Knowing the OMA value is helpful in predicting the natural and anthropogenic stresses to which fish species are exposed [24].

The OMA and otolith absolute mass asymmetry of *Squalius* sampled from ten different freshwater sources in Samsun province were found to be similar between locations ($P>0.05$). Otolith mass asymmetry could potentially result from various environmental stressors, accumulation issues within otoliths, as well as the crystal structures of otoliths (such as aragonitic or vateritic), diseases, genetic predispositions, or a combination of these factors [20].

In this research, both OMA (x) and absolute otolith mass asymmetry ($|x|$) were evaluated in relation to the total length for *Squalius* sp. populations. There is no relationship between fish size and OMA in eight locations for asteriscus otolith and six locations for lapillus otolith. The absence of a relationship may be due to the sample size being small in the study, the samples having the same size range or not differing significantly in terms of size, and possible endogenous reasons [17]. Lychakov et al. [17] were found the relationship between otolith mass asymmetry and fish length in *Sciaenops ocellatus* to be significant, but the r^2 was low. The relationship between fish length and OMA is thought to be associated with a complex trend [29, 51].

This study is expected to contribute to future research on otolith mass asymmetry in freshwater fish species and provide researchers from Türkiye and abroad with the opportunity to compare otolith mass asymmetry among different populations of *Squalius* sp.

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References

[1] Popper, A.N. and Lu, Z., Structure–function relationships in fish otolith organs, **Fisheries Research**, 46, 1-3, 15-25, (2000).

[2] Popper, A.N., Fay, R.R., Platt, C. and Sand, O., Sound detection mechanisms and capabilities of teleost fishes, **Sensory Processing In Aquatic Environments**, 3-38, (2003).

[3] Quist, M.C., Pegg, M.A. and DeVries, D.R., **Age and growth**, in Zale, A.V., Parrish, D.L. and Sutton, T.M., Fisheries techniques, 3rd edition, American Fisheries Society, 677-731, Bethesda, Maryland, (2012).

[4] Schulz-Mirbach, T., Ladich, F., Plath, M. and Heß, M., Enigmatic ear stones: what we know about the functional role and evolution of fish otoliths, **Biological Reviews**, 94, 2, 457-482, (2019).

[5] Campana, S.E., **Photographic atlas of fish otoliths of the Northwest Atlantic Ocean (No. 133)**, NRC Research Press, (2004).

[6] Tuset, V.M., Lombarte, A. and Assis, C.A., Otolith atlas for the western Mediterranean, north and central eastern Atlantic, **Scientia Marina**, 72, S1, 7-198, (2008).

[7] Kennedy, B.P., Klaue, A., Blum, J.D., Folt, C.L. and Nislow, K.H., Reconstructing the lives of fish using Sr isotopes in otoliths, **Canadian Journal of Fisheries and Aquatic Sciences**, 59, 6, 925-929, (2002).

[8] Koeberle, A.L., Arismendi, I., Crittenden, W., Leer, D. and Noakes, D.L., Fluctuating asymmetry of adult Chinook Salmon (*Oncorhynchus tshawytscha*) otoliths from wild and hatchery origins, **Aquatic Ecology**, 54, 1, 431-446, (2020).

[9] Gao, S., Zhang, X., Shu, R., Zhang, S., Lu, J. and Fu, G., Bilateral fluctuation asymmetry of otoliths of *Collichthys lucidus* in different functional areas of Haizhou Bay, **Journal of Fish Biology**, 103, 3, 507-515, (2023).

[10] Harvey, J.T., Loughlin, T.R., Perez, M. A. and Oxman, D.S., Relationship between fish size and otolith length for 63 species of fishes from the eastern North Pacific Ocean, NOAA Technical ReportNMFS, A Technical Report of the Fishery Bulletin, (2000).

[11] Schulz-Mirbach, T. and Plath, M., All good things come in threes–species delimitation through shape analysis of saccular, lagenar and utricular otoliths, **Marine and Freshwater Research**, 63, 10, 934-940, (2012).

[12] Jawad, L. and Qasım, A., Otolith mass asymmetry in *Acanthopagrus arabicus* Iwatsuki, 2013 collected from the Iraqi marine waters, **Bollettino del Museo Civico di Storia Naturale di Verona, Botanica Zoologia**, 45, 33-39, (2021).

[13] Lychakov, D.V. and Rebane, Y.T., Fish otolith mass asymmetry: morphometry and influence on acoustic functionality, **Hearing Research**, 201, 1-2, 55-69, (2005).

[14] Mille, T., Mahe, K., Villanueva, M. C., De Pontual, H. and Ernande, B., Sagittal otolith morphogenesis asymmetry in marine fishes, **Journal of Fish Biology**, 87, 3, 646-663, (2015).

[15] Quindazzi, M.J., Gaffney, L.P., Polard, E., Bohlender, N., Duguid, W. and Juanes, F., Otolith mineralogy affects otolith shape asymmetry: a comparison of hatchery and natural origin Coho salmon (*Oncorhynchus kisutch*), **Journal of Fish Biology**, 102, 4, 870-882, (2023).

[16] Grønkjær, P. and Sand, M.K., Fluctuating asymmetry and nutritional condition of Baltic cod (*Gadus morhua*) larvae, **Marine Biology**, 143, 191-197, (2003).

[17] Lychakov, D.V., Rebane, Y.T., Lombarte, A., Fuiman, L.A. and Takabayashi, A., Fish otolith asymmetry: morphometry and modeling, **Hearing Research**, 219, 1-2, 1-11, (2006).

[18] Lychakov, D.V., Rebane, Y.T., Lombarte, A., Demestre, M. and Fuiman, L.A., Saccular otolith mass asymmetry in adult flatfishes, **Journal of Fish Biology**, 72, 10, (2008).

[19] Jawad, L.A. and Sadighzadeh, Z., Otolith mass asymmetry in the mugilid fish, *Liza klunzingeri* (Day, 1888) collected from Persian Gulf near Bandar Abbas, **Anales de Biología,** 35, 105-107, (2013).

[20] Bostancı, D., Kontaş, S., Yedier, S., Kurucu, G. and Polat, N., Otolith mass asymmetry of *Barbus tauricus* and *Capoeta banarescui* inhabiting Melet River, Ordu, Turkey, **International Ecology Symposium**, 11-13, (2017).

[21] Bostancı, D., Türker, D., Yedier, S., Kontaş, S. ve Kurucu, G., Kuzey Ege Denizi Edremit Körfezi'nde yaşayan sarı kuyruk istavrit, *Trachurus mediterraneus* (Steindachner 1868)'un otolit karakterlerinde dalgalı asimetrinin incelenmesi, **Ordu Üniversitesi Bilim ve Teknoloji Dergisi**, 8, 1, 69-78, (2018).

[22] Mejri, M., Trojette, M., Allaya, H., Ben Faleh, A., Jmil, I., Tazarki, M., Chalh, A., Quignard, J.P. and Trabelsi, M., Stock discrimination of two local populations of *Pagellus erythrinus* (Actinopterygii, Sparidae, Perciformes) in Tunisian waters by analysis of otolith shape, **Cahiers de Biologie Marine**, 59, 6, 579-587, (2018).

[23] Yedier, S., Bostancı, D., Kontaş, S., Kurucu, G. and Polat, N., Comparison of otolith mass asymmetry in two different *Solea solea* populations in Mediterranean Sea, **Ordu Üniversitesi Bilim ve Teknoloji Dergisi**, 8, 1, 125-133, (2018).

[24] Kontaş, S., Bostancı, D. ve Polat, N., Aşağı Melet Irmağı (Ordu, Türkiye)'nda yaşayan *Barbus tauricus* Kessler, 1877 otolit kütle asimetrisinin belirlenmesi, **Journal of Limnology and Freshwater Fisheries Research**, 5, 3, 197-203, (2019).

[25] Kurucu, G., Bostancı, D. ve Polat, N., Aşağı Melet Irmağı (Ordu, Türkiye)'nda yaşayan siraz balığı (*Capoeta banarescui*)'nın otolit kütle asimetrisinin belirlenmesi, **Journal of Anatolian Environmental and Animal Sciences**, 4, 2, 151-155, (2019).

[26] Labidi, M.B., Mejri, M., Shahin, A.A., Quignard, J.P., Trabelsi, M. and Faleh, A.B., Otolith fluctuating asymmetry in *Boops boops* (Actinopterygii, Sparidae) from two marine stations (Bizerte and Kelibia) in Tunisian waters, **Journal of the Marine Biological Association of the United Kingdom**, 100, 7, (2020).

[27] Bouriga, N., Mejri, M., Dekhil, M., Bejaoui, S., Quignard, J.P. and Trabelsi, M., Investigating otolith mass asymmetry in six benthic and pelagic fish species (Actinopterygii) from the Gulf of Tunis, **Acta Ichthyologica et Piscatoria**, 51, 2, 193-197, (2021).

[28] Özpiçak M., Saygın, S. ve Yılmaz, S., Altınkaya Barajı ve Bafra Balık Gölleri (Samsun, Türkiye)'nden örneklenen sudak, *Sander lucioperca* (Linnaeus, 1758)

balığının otolit kütle asimetrisinin incelenmesi, **Ege 9th International Conference On Applied Sciences**, 588-597, (2023).

[29] Özpiçak, M. and Saygın, S., Investigation of otolith mass asymmetry in three stocks of European sardine, *Sardina pilchardus* (Walbaum, 1792) from Türkiye, **Ege Journal of Fisheries and Aquatic Sciences**, 40, 3, 195-200, (2023).

[30] Saygın, S. ve Özpiçak, M., Türkiye denizlerinde (Ege, Karadeniz ve Akdeniz) yayılış gösteren barbunya balığının (*Mullus barbatus*, Linnaeus, 1758) otolit kütle asimetrisi, **Ege 8th International Conference On Applied Sciences** , 506-516, (2023).

[31] Stout, C.C., Tan, M., Lemmon, A.R., Lemmon, E.M. and Armbruster, J.W., Resolving Cypriniformes relationships using an anchored enrichment approach, **BMC Evolutionary Biology**, 16, 1-13, (2016).

[32] Geldiay, R. ve Balık, S., **Türkiye tatlısu balıkları (VI. Baskı)**, Ege Üniversitesi Su Ürünleri Fakültesi Yayınları, (2009).

[33] Turan, D., Yilmaz, B. T. and Kaya, C., *Squalius kottelati*, a new cyprinid species (Teleostei: Cyprinidae) from Orontes River, Turkey, **Zootaxa**, 2270, 53-62, (2009).

[34] Bayçelebi, E., Türkiye'de dağılım gösteren *Squalius* cinsinin taksonomik revizyonu, Doktora Tezi, Recep Tayyip Erdoğan Üniversitesi, Fen Bilimleri Enstitüsü, Rize, (2019).

[35] Sanjur, O.I., Carmona, J.A. and Doadrio, I., Evolutionary and biogeographical patterns within Iberian populations of the genus *Squalius* inferred from molecular data, **Molecular Phylogenetics and Evolution**, 29, 1, 20-30, (2003).

[36] Stefanova, E., Uzunova, E., Hubenova, T., Vasileva, P., Terziyski, D. and Iliev, I., Age and growth of the chub *Leuciscus cephalus* L., from the Maritz River (South Bulgaria), **Bulgarian Journal of Agricultural Science**, 14, 2, 214-220, (2008).

[37] Almada, V. and Sousa-Santos, C., Comparisons of the genetic structure of *Squalius* populations (Teleostei, Cyprinidae) from rivers with contrasting histories, drainage areas and climatic conditions based on two molecular markers, **Molecular Phylogenetics and Evolution**, 57, 2, 924-931, (2010).

[38] Pompei, L., Carosi, A., Pedicillo, G., Rocchini, E. and Lorenzoni, M., Age and growth analysis of the chub, *Squalius squalus* (Bonaparte, 1837), in the Assino Creek (Umbria, Italy), **Knowledge and Management of Aquatic Ecosystems**, 400, 09, (2011).

[39] Ünver, B. and Erk'akan, F., Diet composition of chub, *Squalius cephalus* (teleostei: Cyprinidae), in Lake Tödürge, Sivas, Turkey, **Journal of Applied Ichthyology**, 27, 6, 1350-1355, (2011).

[40] Perea, S., Sousa-Santos, C., Robalo, J. and Doadrio, I., Multilocus phylogeny and systematics of Iberian endemic *Squalius* (Actinopterygii, Leuciscidae), **Zoologica Scripta**, 49, 4, 440-457, (2020).

[41] Doadrio, I., Sousa-Santos, C. and Perea, S., Description of two new species of the genus *Squalius* Bonaparte, 1837 (Actinopterygii, Leuciscidae) in the Iberian Peninsula, **Graellsia**, 79, 2, e205, (2023).

[42] Al Balushi, A.H., Jawad, L.A. and Al Busaidi, H.K., Otolith mass asymmetry in *Lutjanus ehrenbergii* (Peters, 1869) collected from the Sea of Oman, **International Journal of Marine Science**, 7, 37, 366-370, (2017).

[43] Jawad, L., Gnohossou, P. and Tossou, A.G., Bilateral asymmetry in the mass and size of otolith of two cichlid species collected from Lake Ahémé and Porto-Novo Lagoon (Bénin, West Africa), **Anales de Biología**, 42, 9-20, (2020).

[44] Jawad, L. A. and Adams, N.J., Otolith mass asymmetry in the Australian anchovy *Engraulis australis* (White, 1790) predated by Australasian gannets *Morus serrator* (Gray, 1843), Hauraki Gulf, New Zealand, **Cahiers de Biologie Marine**, 63, 4, 371, (2022).

[45] Jawad, L. A., Al-Mamry, J. M., Al-Mamary, D. and Al-Hasani, L., Study on the otolith mass asymmetry in *Lutjanus bengalensis* (Family: Lutjanidae) collected from Muscat City on the Sea of Oman, **Journal of FisheriesSciences.com**, 6, 1, 74, (2012).

[46] Jawad, L.A., Mehanna, S.F., El-Regal, M.A.A. and Ahmed, Y.A., Otolith mass asymmetry in two parrotfish species, *Chlorurus sordidus* (Forsskål, 1775) and *Hipposcarus harid* (Forsskål, 1775) from Hurghada, Red Sea Coast of Egypt, **International Journal of Marine Science**, 7, (2017).

[47] Jawad, L.A., Dörtbudak, M.Y., Yalçin, H. and Park, J.M., Bilateral Asymmetry in Asterisci Otoliths of *Cyprinion kais* and *C. macrostomum* (Cypriniformes, Cyprinidae) collected from Tigris River, Şirnak Region, Türkiye, **Zoodiversity**, 57, 5, 411-420, (2023).

[48] Grønkjær, P., Otoliths as individual indicators: a reappraisal of the link between fish physiology and otolith characteristics, **Marine and Freshwater Research**, 67, 7, 881-888, (2016).

[49] Izzo, C., Reis-Santos, P. and Gillanders, B. M., Otolith chemistry does not just reflect environmental conditions: A meta-analytic evaluation, **Fish and Fisheries**, 19, 3, (2018).

[50] Munday, P.L., Hernaman, V., Dixson, D.L. and Thorrold, S.R., Effect of ocean acidification on otolith development in larvae of a tropical marine fish, **Biogeosciences**, 8, 1631-1641, (2011).

[51] Jawad, L.A., Al-Mamry, J.M., Al-Mamari, H.M., Al-Yarubi, M.M., Al-Busaidi, H. K. and Al-Mamary, D.S., Otolith mass asymmetry in *Rhynchorhamphus georgi* (Valenciennes, 1846) (Family: Hemiramphidae) collected from the Sea of Oman, **Journal of Black Sea/Mediterranean Environment**, 17, 1, 47-55, (2011).