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BILATERAL ASYMMETRY IN ASTERISCI OTOLITHS OF *CYPRINION KAIS* AND *C. MACROSTOMUM* (CYPRINIFORMES, CYPRINIDAE) COLLECTED FROM TIGRIS RIVER, ŞIRNAK REGION, TÜRKİYE

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Bilateral Asymmetry in Asterisci Otoliths of *Cyprinion kais* and *C. macrostomum* (Cypriniformes, Cyprinidae) collected from Tigris River, Şirnak Region, Türkiye. Jawad, L. A., Dörtbudak, M. Y., Hamza Yalçin, H. & Park, J. M. — Otolith traits such as size and mass were investigated in 60 fish specimens of *C. kais* and *C. macrostomum* collected from the Tigris River, Şırnak region, Türkiye collected from September 2015 to December 2015. The purpose of this work was to determine the asymmetry level in the otolith length (OL), otolith width (OW), and otolith mass (OM). OL's rate of asymmetry was greater than OW's and OM's. The asymmetry in the three otolith traits rose with the fish length. The plausible reason for asymmetry in the otolith features explored has been considered relative to the inconsistency of growth triggered by ecological influence associated with the inconsistency in water temperature, salinity, depth, and contaminants occurring in the Tigris River System.
Key words: Actinopterygii, Cyprinidae, ecological factors, *Cyprinion kais*, *Cyprinion macrostomum*.

Introduction

In the inner ear of fish, there are bony constructions known as otoliths (Popper & Lu, 2000). These structures are helpful in fish identification, evolutionary studies, and biological and fisheries management studies, where the age of the fish can be determined based on ageing annuli found on the body of the otoliths (Jawad & Mahé, 2022). The otoliths are inert metabolically and show continuous development all through the life of the fish (Casselman, 1987). Their specific shape is usually used in separating species and fish stocks. The inner ear inside the head of the fish accommodates three pairs of otoliths: sagittae, asterisci, and lapilli (Wright et al., 2002). In most teleost fish species, the sagittae are the more prominent, and the asterisci are the smaller, but in Cypriniform species, the asterisci are the larger (Phelps et al., 2007). Therefore, researchers usually used sagittae (Panfili et al., 2002).

In latest years, the external features of the otoliths, precisely their size, have been believed to be a means to assess different impacts (for review, see Ben Labidi et al., 2020). Investigations above have disclosed that the otolith morphology is one of the pinpointing qualities of the species (Sadighzadeh et al., 2014) and influences alter that discrepancy in the otolith development during this development (Vignon and Morat, 2010; Vignon, 2015; Hüssy et al., 2016; Ider et al., 2017; Fashandi et al., 2019). Even so, the likely cause for the intra-individual inconsistency, primarily the asymmetry in the shape of the otolith on both sides of the fish head, has been insufficiently explored (Mille et al., 2015). In a healthy habitat, the growth of the three otoliths in the fish head is symmetrical regarding their external features (Panfili et al., 2002). Yet, between individuals of the species, deviations in the structure of the otolith are perceived (Popper & Lu, 2000) also mass differences (Ambuali et al., 2011; Jawad et al., 2012; Jawad et al., 2017; Jawad et al., 2020; Jawad, 2013; Jawad and Sadighzadeh, 2013; Al Balushi et al., 2017; Dkhili et al., 2017; Yedier et al., 2018). This irregularity in mass asymmetry is assumed to unveil the growth anomalies of fish prompted by varied types of pressure, such as genetic or ecological impacts (Grønkjaer & Sand, 2003), and it might be immense (Scherer et al., 2001) or trivial (Takabayashi & Ohmura-Iwasaki, 2003). Physiologically, the rise or fall in the asymmetry value of the otolith mass can negatively interrupt other biological issues essential to the endurance of the fish, especially the hearing, equilibrium, and linear speeding up (Panfili et al., 2005; Yedier et al., 2018).

The Kais kingfish, *Cyprinion kais,* is a freshwater species that lives in the Benthopelagic region (Froese & Pauly, 2022). Individuals of this species reach a maximum total length of 159 mm and a maximum recorded weight of 49.90 g (Esmaeili et al., 2014 a). They feed on filamentous algae, aquatic insects, and detritus (Al-Rudainy, 2008). They reach maturity at age two, with females spawning over sand, stones, and gravel in May–June. This species is restricted in its distribution to areas specializing in flowing water, and they move to canals and other aquatic habitats for feeding. It is widespread in the Tigris–Euphrates Rivers basin, representing several separate populations (Ünlü, 2006).

The Tigris kingfish, *Cyprinion macrostomum,* is a freshwater species that lives in a benthopelagic region (Froese & Pauly, 2022). Individuals of this species reach a maximum total length of 204 mm and a maximum recorded weight of 91.20 g (Esmaeili et al., 2014 b). Some characteristic features separate mature females from the males, such as the presence of large tubercles on the snout in a broad band below the nostril level of the males, extending under the eye and breaking into a few tubercles on the operculum. In addition, there is an apparent tuberosity between the nostril and the eye, and fine tubercles are dispersed over the top of the head (Coad, 2012). This species is found in the Orontes (= Asi), Quwayq Rivers, and the Tigris-Euphrates basins. It prefers living in rivers, streams, lakes, dams, lagoons, ponds, springs, marshes, canals, jubes (= irrigation ditches), and gravel pits (Pirani et al., 2013). The maximum known age of this species is five years (Faghani Langroudi & Mousavi Sabet, 2018). It reaches maturity at 100–111 mm (Allouse et al., 1986). It feeds mainly on periphyton, including *Navicula*, *Cymbellla*, *Diatoma*, and *Nitzschia* (Marammazi et al., 2014). Spawning happened from late May to mid-August when the water temperature was 16–24 °C (Faghani Langroudi & Mousavi Sabet, 2018).

The influence of environmental impacts on the otolith morphology has been discussed by several studies that proposed that environmental factors such as water temperature, salinity, food availability, depth, and habitat type are the principal matters that are predictable to be responsible for the variations in otolith morphology among related individuals (Gagliano & McCormick, 2004; Mérigot et al., 2007; Hüssy, 2008; Morat et al., 2012). The most recent investigations in this field on Turkish shores have specified that the otolith morphology is likely to be associated with the discrepancy in ecological sites (Kontaş et al., 2017; Bostancı et al., 2017; Kontaş et al., 2018; Kurucu et al., 2019; Yedier et al., 2022; Reis & Ateş, 2022). Although much research has been conducted, the irregularity of the otolith morphology and mass in the two *Cyprinion* species examined in the existing study is lacking in Turkish waters. Consequently, this research aims to establish the level of the otolith mass, length, and width asymmetry for the two *Cyprinion* species chosen for the existing investigation, which were collected from the Tigris River at Şırnak region, Türkiye, and its range was calculated. Likewise, the disparity of this asymmetry was estimated through the different lengths of the examined specimens.

Material and Methods

Sixty specimens (30 specimens for each of *C. kais* and *C. macrostomum*) were attained through a routine ichthyological collection in the Tigris River. The fishing activity was performed by the first author at the Şırnak region between Güçlükonak and Cizre from September 2015 to December 2015 (fig. 1). Fish specimens of both species examined (fig. 2) were kept chilled by using an ice box till arriving at the laboratory and were identified following Coad (2022). Later on, the fish specimens were measured to the nearest mm for total length (TL), standard length (SL), and fork length (FL). After that dissected to remove the asteriscus otolith from both sides of the fish head. As soon as the otoliths were out, they were cleaned in 70 % ethanol and dried at room temperature. The otolith length (OL) (the longest most horizontal length across the otolith) and width (OW) (the most extended vertical length across the otolith) were determined using a digital calliper with an accuracy of 0.01 mm (fig. 3). Total otolith mass (OM) was weighed using a normal analytical scale (HR-250AZ, A&D Company Ltd) to the nearest 0.0001 g after Harvey et al. (2000) and Battaglia et al. (2010). Statistical analyses were performed according to the squared coefficient of asymmetry variation (CV_a^2) for the length, width, and mass of the otolith obtained based on Valentine et al. (1973):

$$CV_a^2 = (S_{r-l} \text{ X } 100/X_{r+l})^2, \text{ where:}$$

S_{r-l} = the standard deviation of signed variances, X_{r+l} = is the mean of the character, calculated by adding the absolute scores for both sides and dividing by the sample size.

Fig. 1. Map showing the location of sampling.

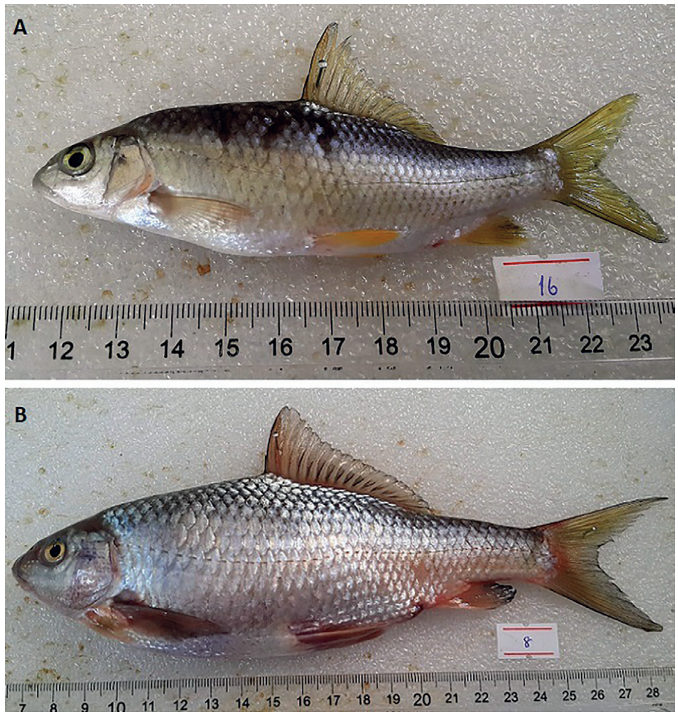

Fig. 2. A, *Cyprinion kais*, 179 mm TL; B, *C. macrostomum*, 190 mm TL.

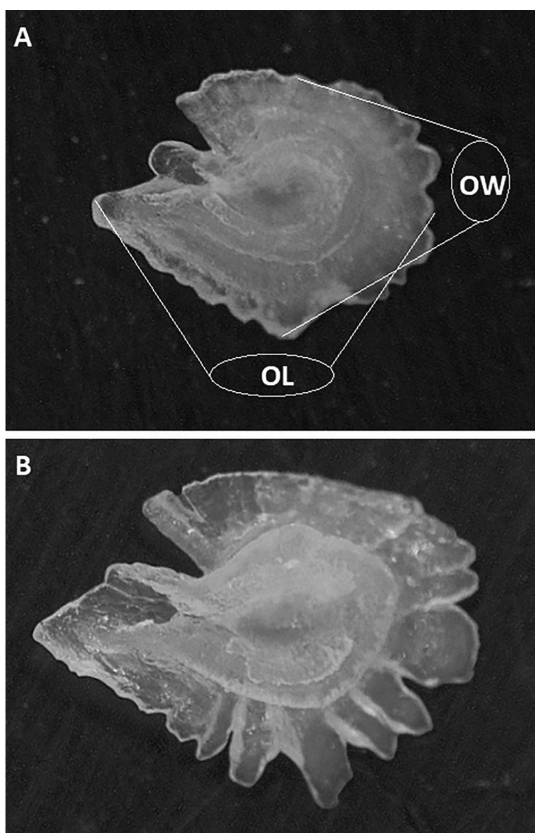

Fig. 3. Asteriscus otolith of A, *Cyprinion kais*, 179 mm TL; B, *C. macrostomum*, 190 mm TL. OL = otolith; OW = otolith width.

Individual errors in measuring sizes and otolith mass can disguise the results of dissimilarity calculations that render them useless (Palmer, 1994). Thus, to elude any unwanted fault in the present study, the sizes and mass of the otolith were recorded by a single reader and achieved two times (Lee & Lysak, 1990). The average measurements and mass of the otolith were used in this analysis. Dissimilarity coefficients were calculated between the several length groups using the ANOVA test. The X values of the otolith mass asymmetry vary from −2 to +2. These limit values imply maximal asymmetry, while the '0' value signifies no asymmetry between the right and left otoliths. For otolith mass asymmetry, a positive value of "X" means that the left otolith is lighter than the right one ($M_L < M_R$), while a negative value of "*X* "indicates that the left otolith is heavier than the right one ($M_L > M_R$). For each *Cyprinion* species analysed, the experimented individuals were divided into length classes based on total length, and then the fluctuating asymmetry value for each size class was calculated. The ANOVA test assessed the asymmetry coefficients among and within the size classes of the two Cyprinion species.

Results

The analysis of asymmetry estimates of the OL, OW, and OM of *C. kais* and *C. macrostomum* ranging in total length of 115–143 mm and 170–175 mm for *C. kais* and *C. macrostomum,* respectively, were collected from the Tigris River at Şırnak region, Türkiye, are presented in table 1. This study's data on otolith length, width, and mass asymmetry are 63.67, 44.51, and 2.4556 for *C. kais* and 61.45, 43.81, and 2.4252 for *C. macrostomum*.

The OL, OW, and OM asymmetry estimate for *C. kais*) were higher than those attained for *C. macrostomum*. In both species, the value of otolith length asymmetry was the highest among the values of asymmetry obtained for otolith width and otolith mass. The results revealed that the absolute symmetry between left and right otoliths (X = 0) is rare. Generally, X values vary around "0". In this investigation, the percentage of asymmetry exceeded 50 % in the two fish species investigated.

In the fish length groups of the two *Cyprinion* species examined, the highest level of asymmetry of the three otolith biometric parameters (length, width, and mass) was detected in fish with a considerable length of each species (table 1). The results indicated a tendency for a rise in the estimates of the asymmetry of the three otolith characteristics (length, width, and mass) of the two *Cyprinion* species explored (table 1).

The percentage of specimens exhibiting asymmetry in the OL characteristic was the highest among the rates deliberate for the OW and OM of the two *Cyprinion* species studied (table 1). The percentage of individuals with asymmetry was highest in those belonging to the largest fish length group of each otolith feature assessed (table 2).

Table 1. The squared coefficient of asymmetry (CV^2_a) value and character means (Xr+l) of two *Cyprinion* fish species, *Cyprinion kais* and *C. macrostomum*, collected from Tigris River, Şırnak region, Türkiye

Character	CV^2_a	N	Character mean (mm) ± SD	% of individuals with asymmetry
			Cyprinion kais	
Otolith length	63.67	30	10.05 ± 1.23	87
Otolith width	44.51	30	9.18 ± 1.02	77
Otolith mass	2.4556	30	16 ± 0.98	54 %
			Cyprinion macrostomum	
Otolith length	61.45	30	12.19 ± 0.11	62
Otolith width	43.81	30	10.48 ± 0.13	55
Otolith mass	2.4252	30	22 ± 0.97	34

Table 2. The squared coefficient of asymmetry (CV^2_a) and character means (otolith length, width in mm, and mass in g) by the size of two *Cyprinion* fish species, *Cyprinion kais* and *C. macrostomum* collected from Tigris River, Şırnak region, Türkiye

Character	CV^2_a	N	Characters mean	% of individuals with asymmetry
			Cyprinion kais	
Otolith length		30		
111–130	61.63	18	9.98 ± 1.47	87
131–150	64.81	12	10.03 ± 2.03	83
Otolith width		30		
111–130	41.81	18	8.99 ± 1.03	53
131–150	44.42	12	9.01 ± 2.05	56
Otolith mass		30		
111–130	2.4375	18	16.11 ± 2.53	86
131–150	2.4578	12	15.98 ± 1.56	78
			Cyprinion macrostomum	
Otolith length		30		
131–170	61.25	15	12.01 ± 1.44	77
171–200	62.65	13	12.32 ± 2.31	84
201–240	63.41	2	11.99 ± 2.11	87
Otolith width		30		
131–170	40.31	15	10.35 ± 2.01	55
171–200	42.54	13	10.42 ± 2.25	57
201–240	43.72	2	9.98 ± 2.33	65
Otolith mass		30		
131–170	2.4287	15	21.98 ± 1.65	57
171–200	2.4329	13	21.87 ± 2.04	65
201–240	2.4428	2	22.03 ± 1.20	78

Note. N — number of specimens; SD — standard deviation.

Discussion

In the present examination of the three characteristics of the asterisci of the two *Cyprinion* species collected from the Tigris River, Şırnak region, Türkiye was performed to exhibit the bilateral asymmetry in these features. The recognised asymmetry in the otolith features of the asteriscus of the two *Cyprinion* assessed may lessen the capability of the young individuals to stay on and be present in their suitable habitats (Gagliano et al., 2008). This defect will lead to the imprecision of realising their home region by the young individuals of the fish species. Regarding commercial fishing, such a crisis will decrease the fish stock of a specific species. While the two *Cyprinion* species investigated are amongst the essential freshwater species of Türkiye, the asymmetry on the otolith of the young will disturb the stock of these species in this area.

The external morphology of the fish is regularly exposed to alterations in the habitat background. Subsequently, the more significant asymmetry estimates of the otolith attributes may indicate such an impact. The data on the link between various ecological contaminations and the otolith aspects of the two *Cyprinion* species investigated in the current work must be included, which consecutively complicates evaluating asymmetry and pollution levels. Hence, it is not expected at this phase to have a specific outcome on the extrapolation of this event. The investigation examined the two Cyprinion species from only one locality. Concurrently, no preceding information takes place on its asymmetry fluctuating to permit judgments and evaluate the fluctuating asymmetry level. The present conclusions will be regarded as a case study for future investigations and a reference record for analogy.

The assessment of the aspects of the otoliths revealed that the maximum level of asymmetry in the two *Cyprinion* species analysed is the OL. Essentially, the asymmetry between the right and left asterisci has previously been acknowledged for certain fish species (*Rastrelliger kanagurta* (Cuvier, 1817) (Al-Mamry et al., 2011); and *Carangoides caerulepinnatus* (Rüppell, 1830) (Jawad, 2013) for which information is available. This could keep the exposure of the otolith length to instantaneous alterations in the living surroundings. Furthermore, the OM displayed the lowermost bilateral asymmetry level in the two *Cyprinion* species explored, which means that this trait might be less subjected to ecological stress effects involving pollution. Then, this minor asymmetry level can be interpreted as the emerging phase of the otolith mass that might not be related to unfavourable environmental surroundings (Jawad, 2003).

Preceding research on otoliths disclosed several benthic and pelagic species with a mean level of otolith mass asymmetry from −0.2 to 0.2 (Lychakov et al., 2008; Jawad & Sadighzadeh, 2013; Al Balushi et al., 2017). The investigation revealed a higher otolith mass asymmetry level for the two Cyprinion species analysed. This result concurs with that of Bouriga et al. (Bouriga et al., 2021) on *Trachurus mediterraneaus* achieved from different localities on the Tunisian waters of the Mediterranean Sea. Bouriga et al. (2021) related such high values to the physiological condition of these species, their niches, and ecological impacts (abiotic and biotic), where environmental and anthropogenic pressures have a notable influence on the development of otoliths (Grønkjær, 2016).

As previously shown by Helling et al. (2003), the apparent effect of bilateral asymmetry in fish otoliths is unusual swimming activity and disturbance with accurate sound signals, resulting in the individuals' inability to participate in their habitat (Lychakov & Rebane, 2005). Likewise, in comparison with the outcome accomplished in the research at hand, equivalent and discrepancy results have been reached by various investigators for several fish species across the world. For instance, Mejri et al. (2020) explored asymmetry in the otolith shape, length, width, and area in *Pagellus erythrinus* (Linnaeus, 1758) obtained from the Gulf of Tunis. They found intra- and inter-population asymmetry is present in these characteristics. Jawad (2012) suggested that the asymmetry rate in the otolith width is at its peak amongst the length and width data gained for *Lutjanus bengalensis* (Bloch, 1790) obtained from the Muscat coast on the Sea of Oman. Similarly, Jawad et al. (2012) in *Sar*dinella sindensis (Day, 1878) and Sillago sihama (Forsskål, 1775) attained from the Persian Gulf near Bandar Abbas and Jawad et al. (2020) in *Sarotherodon melanotheron* Rüppell, 1852, and *Coptodon guineensis* (Günther, 1862) inspected from Lake Ahémé and Porto-Novo Lagoon, Bénin, reached to a similar outcome. Likewise, Kontaş et al. (2018) assessed the fluctuating asymmetry of the otolith area, length, perimeter, and width in four groups of *Merlangius merlangus* (Linnaeus, 1758) gained from the Middle Black Sea. They discovered that the otolith area had the topmost asymmetry while the length had the bottommost. Also, they recommended no significant relationship between the asymmetry levels of the four otolith characters and the total length. The asymmetry in these features could result from the strain of various Black Sea impurities.

Then, pollution has become a normal phenomenon for the two *Cyprinion* species living in the basin of the Tigris River and may be held responsible for the asymmetry observed in the three otolith characteristics, otolith length, width, and mass, and in particular in the otolith length. In addition, various authors have anticipated that genetic issues may be blamed for the asymmetry on both sides of the otolith (Panfili et al., 2005). Yet, this issue cannot be considered here because of the absence of genetic data on the two Cyprinion species analysed.

Conflict of interest

The authors state that there are no conflicts of interest.

Ethical statement

This work is based on specimens collected through a routine ichthyological survey by the first author, and the fish specimens obtained from the collection sites were dead. Therefore, no ethics is required in this case.

Author contribution

All authors contributed to the study's conception and design. Material preparation and data collection were performed by (Muhammed Yaşar Dörtbudak), and the statistical analyses were accomplished by (Hamza Yalçin) and (Joo Myun Park). The first draft of the manuscript was written by (Laith A. Jawad), and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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