

Investigation of asymmetry in sagitta otolith features in relation to total length in *Lethrinus* species from the Red Sea coast of Yemen

by

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Abstract

This study aimed to investigate the asymmetry in otolith length (OL), otolith width (OW) and otolith weight (OWe) in relation to total body length in 224 fish samples of *Lethrinus borbonicus*, *L. letnjan* and *L. mahsena* collected from the Red Sea coast of the Republic of Yemen. The results showed that the irregularity of OWe was greater than that of OL and OW. The degree of disproportionality in the three otolith features increased with the growth in the fish size. The likely reason for the asymmetry in the otolith parameters explored has been discussed in relation to the inconsistency in development provoked by territorial influence linked to the discrepancies in water temperature, salinity, depth, and impurities found in the southern Red Sea water system.

Key words: Actinopterygii, abiotic factors, *Lethrinus borbonicus*, *Lethrinus letnjan*, *Lethrinus mahsena*, Lethrinidae, environmental factors

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1. Introduction

The three lethrinid species, *Lethrinus borbonicus*, *L. letnjan* and *L. mahsena*, are marine and live in association with coral reefs (Lieske & Myers, 1994). The maximum total length reported for *L. borbonicus* is 400 mm, with a common total length of 250 mm for male/unsexed individuals (Carpenter & Allen, 1989). This species is distributed in the Indian Ocean region, where it has been reported from the Red Sea and the Arabian Gulf south to Durban, South Africa, then east to North Bay Reef, Andaman and Nicobar Islands (Carpenter & Allen, 1989). Individuals of this species live in sandy areas near reefs during the daytime, sometimes in small groups. At night, they are solitary and range over reef-flats and slopes where they feed primarily on echinoderms, molluscs, and crustaceans (Carpenter & Allen, 1989). This species exhibits juvenile hermaphroditism (Ebisawa, 2006).

Lethrinus letnjan is non-migratory and lives at a depth range of 10–90 m (Kailola et al., 1993). It reaches a maximum total length of 520 mm (Toor, 1964), with a common total length of 400 mm (Bouhleb, 1988). The maximum reported age for this species is 19 years (Grandcourt, 2002). It is widespread in the Indo-West Pacific region from the Red Sea, Arabian Gulf, and East Africa to the Ryukyus and Tonga. Individuals of this species are reported to inhabit sandy bottoms in coastal areas, deep lagoons, and near coral reefs (Sommer et al., 1996). Juveniles and small adults are commonly found in loose aggregations over seagrass beds, mangrove swamps and shallow sandy areas, while adults are generally solitary in deeper waters. They feed primarily on crustaceans and molluscs, but they also consume echinoderms, polychaetes, and fishes in considerable quantities (Carpenter & Allen, 1989). They are protogynous hermaphrodites (Allsop & West, 2003). They are primarily caught by handline, traps, trawls, beach seines, and gill nets and are usually sold fresh (Carpenter & Niem, 2001).

The species *L. mahsena* is non-migratory and inhabits a depth range of 2–100 m (Lieske & Myers, 1994). It reaches a maximum total length of 650 mm (Carpenter & Allen, 1989), with a common total length of 400 mm (Carpenter & Allen, 1989). The species is reported to live up to a maximum of 27 years (Grandcourt, 2002). It is distributed in the Indian Ocean and has been reported from the Red Sea and East Africa to Sri Lanka.

The waters of the Red Sea coast of the Republic of Yemen are reported to be heavily polluted. Al-qadasy et al. (2017) investigated the concentrations of toxic metals, lead and cadmium in water, sediments, and fish organs (muscle, liver, and gill). Their results

showed that the lead concentration in seawater is 0.045–0.055 mg · L⁻¹ and in sediment is 33.512–35.726 µg · g⁻¹ dry weight. The cadmium concentration in seawater is 0.006–0.010 mg · L⁻¹ and in sediment is 1.944–2.004 µg · g⁻¹ dry weight. The lead concentration is in its highest level in most fish gill samples 0.047–0.727 µg · g⁻¹ dry weight, whereas in muscles is the lowest 0.020–0.116 µg · g⁻¹ dry weight, and in liver was 0.038–0.267 µg · g⁻¹ dry weight. Cadmium concentration is in its highest level in most fish gill samples 0.033–0.609 µg · g⁻¹ dry weight, whereas in muscles is the lowest 0.018–0.073 µg · g⁻¹ dry weight, and in liver was 0.028–0.209 µg · g⁻¹ dry weight. Al-qadasy et al. (2017) concluded that pollution levels in Yemen are currently within the lower limits of pollution.

The effects of pollutants on the three lethrinid species examined are lacking. Al-Qadasy et al. (2018) studied the concentrations of heavy metals (Pb, Cd, Hg and As) in water and fish muscle collected from three different sites (Aden, AL-Hudaydah, and AL-Mukalla) of the Red Sea and the Gulf of Aden in Yemen's coastal. Their results revealed that the concentration (mg · L⁻¹) of heavy metals in seawater (Pb-0.061 ± 0.005, Cd-0.007 ± 0.001, Hg-0.007 ± 0.0005, and As-0.008 ± 0.0003) was lower than the concentration (µg · g⁻¹) of heavy metals in muscle (Pb 0.101 ± 0.012, Cd-0.046 ± 0.010, Hg-0.058 ± 0.002, and As-0.089 ± 0.002). Their results also showed that the concentration of heavy metals was high at stations AL-Hudaydah and AL-Mukalla and low at the station in Aden. They concluded that the highest mean concentration of Pb, Cd, and As in the muscles of the four studied fish species was 0.137 ± 0.014 µg · g⁻¹ dry weight and 0.069 ± 0.021 µg · g⁻¹ dry weight in large *Epinephelus areolatus*; 0.106 ± 0.007 µg · g⁻¹ dry weight in large *Lethrinus mahsena* at Site AL-Hudaydah in Summer. Also, the highest mean concentration of Hg in the muscles of the four studied fish species was 0.071 ± 0.012 µg · g⁻¹ dry weight in large *E. areolatus* at Site Aden in summer. They concluded that positive relationships were found between metal levels and fish size. In conclusion, their results showed that the concentration of heavy metals was significantly higher during the summer season in seawater and muscle fish samples at all stations throughout the study period. The study by Ahmed et al. (2024) focused on the detrimental impact of human activities on the coastal ecosystem. They investigated the levels of hazardous metals in the Red Sea coast of Yemen. Their study analysed the average levels of toxic metals—Pb, Cd, Hg, and As—in water, sediments, and various fish organs, including muscle, liver, and gills acquired from AL-Hudaydah city, situated on the Red Sea coast



of Yemen. Their results showed that the level of Pb, Cd, Hg, and As in the sediments were 75.883–77.059, 2.380–2.495, 0.011–0.028, and 0.091–0.109 dry weight, respectively. The levels of toxic metals in fish tissue varied as follows: Pb 0.031–0.357, Cd 0.018–0.146, Hg 0.019–0.082, and As 0.044–0.121 dry weight in muscle; Pb 0.058–1.466, Cd 0.057–0.700, Hg 0.009–0.044, and As 0.011–0.074 dry weight in gills; and Pb 0.049–0.999, Cd 0.047–0.705, Hg 0.051–0.186, and As 0.065–0.198 dry weight in liver cells. They concluded that the pollution level in Yemen is currently higher than the established limits.

There are three pairs of otoliths inside the fish ear. Among these are the sagittae, which are considered the most important structure for fish biologists. Different to bones and scales, the sagittae are metabolically inactive, such that any material deposited endures unchanged and cannot be reabsorbed (Campana & Neilson, 1985). Owing to these two traits, otoliths are absolute black boxes (Lecomte-Finiger, 1999), comprising consistent patterns that are believed to be a vital resource of data for rebuilding a fish's entire life duration (Campana & Thorrold, 2001).

The inconsistency in growth of a bilateral characteristic between the left and right sides of an organism is known as asymmetry (Leary & Allendorf, 1989; Palmer & Strobeck, 1992; Van Vallen, 1962; Palmer, 1994; Fey & Hare, 2008).

Asymmetry of otoliths has been proposed as a useful index of body condition and health (Allenbach, 2011; Grønkaer & Sand, 2003) during the initial growth of fish (Gagliano & McCormick, 2004). Fluctuating asymmetry signifies an explicit outline of bilateral change in a specific feature exhibited by a sample of individuals (Somarakis et al., 1997b). Uneven otoliths might be destructively disturbing the sensory accuracy of the inner ear (Gagliano et al., 2008; Lychakov & Rebane, 2005). Previous investigations have mainly focused on the life of fish larvae (Panfili et al., 2005). A few surveys have revealed that the bigger fish otolith asymmetry that can happen due to traumatic instances instigated by contamination (Franco et al., 2002), parasitism (Escós et al., 1995), and meagre feeding instances (Somarakis et al., 1997a, 1997b). Though, further investigations have perceived a robust link between strain and irregularity in otolith (Fey & Hare, 2008; Folkvord, 2005; Panfili et al., 2005).

Valuation of the degree of asymmetry has not been made on the otolith width (OW) or length of the three lethrinid fish species surveyed in the investigation at hand, where fish specimens were obtained from the Yemeni coast of the Red Sea. This investigation is new for the fish fauna of this part of the world. Here, we

inspect the links between otolith length (OL), width, and weight and the total length of the fish samples from the three species in the Yemeni coast of the Red Sea to enhance the accuracy of their parameterisation for the southern Red Sea area food web and ecosystem studies.

2. Materials and methods

A total of 50 otoliths were extracted from 98 specimens of *L. borbonicus* (154–385 mm TL), 144 otoliths from 72 specimens of *L. Letnjan* (160–492 mm TL), and 108 otoliths from 54 specimens of *L. mahsena* (165–504 mm TL) were examined (Fig. 1. Samples of the three lethrinid species were collected from the Salif (15° 19' 01.06" N 42° 39' 49.80" E), Hudaydah (14° 32' 51.66" N 42° 49' 20.19" E), and Kamaran (15° 17' 54.51" N 42° 37' 39.20" E) for *L. borbonicus*, *L. letnjan*, and *L. mahsena*, respectively (Fig. 2). Fish specimens from the three localities were collected in February 2014 using a gill net operated by a local fisher. The otoliths were extracted from both sides of the fish head by creating an opening on the top of the skull. The otic capsules were separated, and the otoliths were detached with a pair of fine tweezers (Fig. 3).

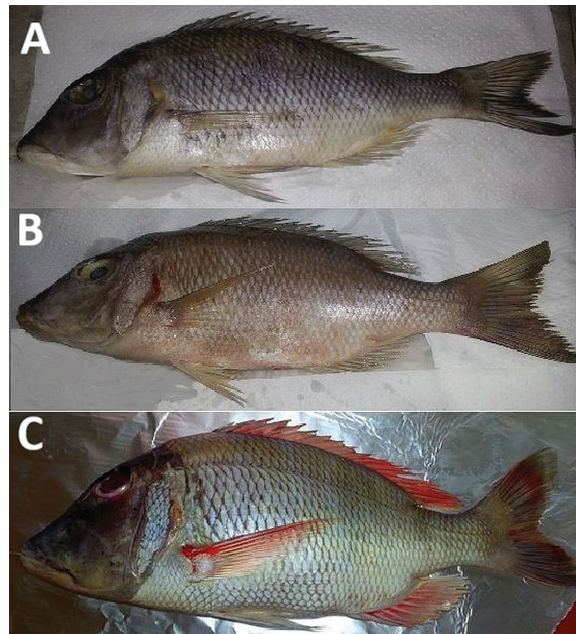
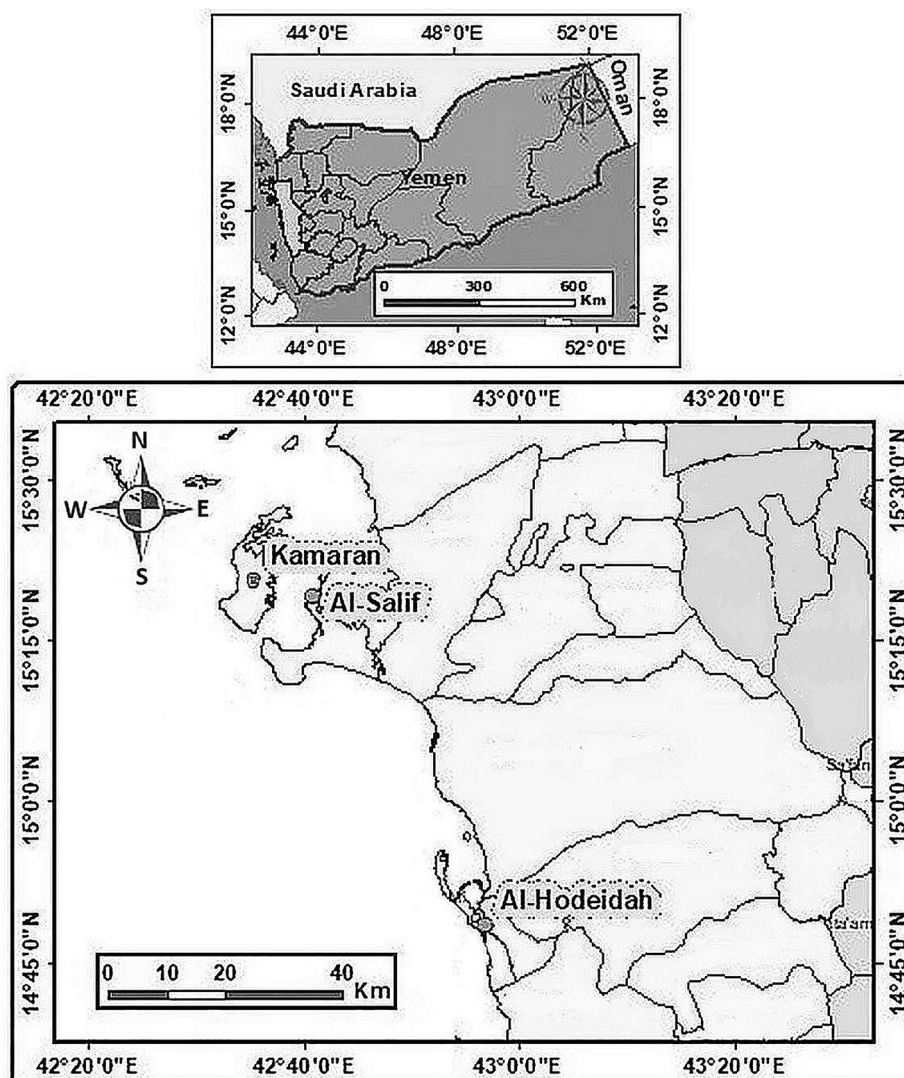


Figure 1

Three species of the family Lethrinidae examined from the Red Sea coast of Yemen. (A) *Lethrinus borbonicus*, 215 mm TL; (B) *Lethrinus lentjan*, 220 mm TL; (C) *Lethrinus mahsena*, 275 mm TL.

**Figure 2**

Map showing the sampling locations of three lethrinid species from the Red Sea coast of Yemen.

They were then washed with 70% ethanol and stored dry. OL and width were determined to the nearest millimetre under an examining microscope. These measurements were taken following the studies of Al-Rasady et al. (2010), Jawad et al. (2012a, 2012b), and Abu El-Regal et al. (2016). Otolith weight (OWe) was measured using a standard analytical scale (NW-137BR, C&B Company Ltd, Oxford, UK) to the nearest 0.0001 mg. According to Valentine et al. (1973), unevenness was computed as the squared coefficient of asymmetry disparity (CV^2_a) for the two otolith sizes:

$$CV(S_{r-1} \times 100 / X_{r+1})^2$$

where S_{r-1} is the standard deviation (SD) of signed changes and X_{r+1} is the mean of the character, which

is calculated by adding the absolute scores for both sides and dividing by the sample size. Irregularity evaluations and measurement errors are generally small and normally distributed around a mean of zero (Merilä & Björklund, 1995). Individual changes in taking measurements can disturb the results of bilateral asymmetry analysis (Palmer, 1994). Accordingly, in this study, all dimensions were obtained by only one investigator to eliminate any undesirable mistake (Lee & Lysak, 1990) and were recorded twice. Coefficients of asymmetry were compared across different total length classes using an ANOVA test. In addition to the ANOVA test, Tukey HSD post hoc test was performed to evaluate whether the modifications between pairwise comparisons of length classes were significant (StatSoft Inc., 1991).



Table 1

Squared coefficient of asymmetry (CV^2_a) value and character means (X_{r+1}) of three lethrinid fish species collected from the Red Sea coast of the Republic of Yemen.

Character	CV^2_a	N	Character mean (mm) \pm SD	% of individuals with asymmetry
<i>Lethrinus borbonicus</i>				
OL	53.54	49	8.39 \pm 1.2	45
OW	55.47	49	7.40 \pm 1.7	92
OWe	75.67	49	0.271 \pm 2.1	76
<i>Lethrinus letnjan</i>				
OL	54.78	72	10.9 \pm 1.8	55
OW	58.55	72	7.89 \pm 1.2	88
OWe	73.74	72	0.338 \pm 1.6	65
<i>Lethrinus mahsena</i>				
OL	51.77	54	7.9 \pm 1.2	71
(OW	57.34	54	6.4 \pm 1.3	89
OWe	71.65	54	0.230 \pm 1.4	87

OL, otolith length; OW, otolith width; OWe, Otolith weight; SD, standard deviation.

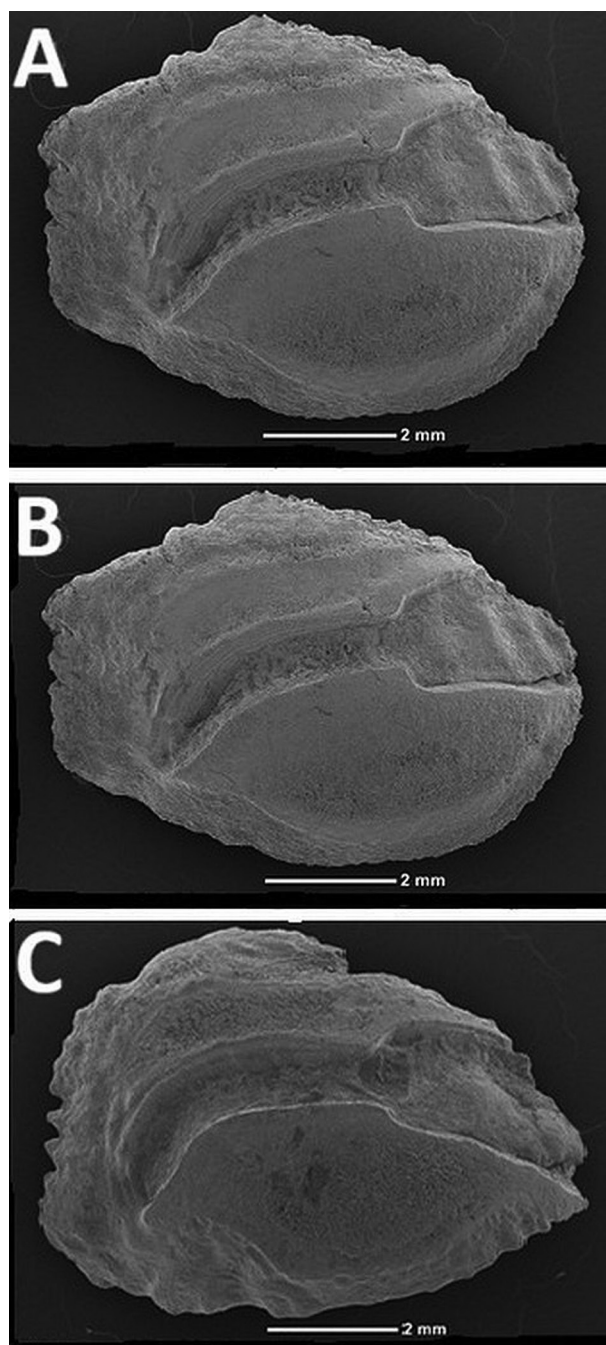


Figure 3

Otolith of (A) *Lethrinus borbonicus*, 230 mm TL; (B) *Lethrinus letnjan*, 227 mm TL; (C) *Lethrinus mahsena*, 281 mm TL.

3. Results

The results of asymmetry investigation of the OL, width, and weight of the three lethrinid species are presented in Table 1. The amount of irregularity of

the OWe was higher than that of OL and weight for all three species. Similarly, the unevenness degrees of length classes were significantly different ($p < 0.05$). For the three otolith features examined in the present work, the results revealed that the grades of irregularity at its lower and peak grades in the fish ranging in TL between 150–200 and 351–400, 160–210 and 451–500, and 160–210 for *L. borbonicus*, *L. letnjan*, and *L. mahsena*, respectively.

The percentage of fish specimens displaying irregularity in the OW trait was at its maximum between the percentages considered for the three otolith characteristics of the three species examined (Table 1). The specimens of *L. borbonicus*, *L. letnjan*, and *L. mahsena* were classified into length classes (Table 2). A propensity for the unevenness grade to upsurge with fish length was observed in the three otolith traits investigated.

4. Discussion

This work was accomplished to investigate the two-sided irregularity in otolith traits of three lethrinid species. Such criterion might decrease the capability of the juvenile specimens to survive and be present in the suitable habitats that they live in Gagliano et al. (2008).

The investigation of the traits of the otoliths revealed that the extreme point of irregularity in

Table 2

Squared coefficient of asymmetry and character means by size class of three lethrinid fish species collected from the Red Sea coast of the Republic of Yemen.

Character	CV ² _a	N	Character mean (mm) ± SD	% of individuals with asymmetry
<i>Lethrinus borbonicus</i>				
OL				
150–200	32.42	10	8.49 ± 1.5	43
201–250	32.87	15	8.55 ± 1.7	44
251–300	33.41	15	7.98 ± 2.1	54
301–350	33.98	5	8.58 ± 2.5	61
351–400	34.12	4	8.72 ± 2.4	65
OW				
150–200	65.59	10	7.73 ± 1.6	89
201–250	65.99	15	7.53 ± 1.5	90
251–300	66.42	15	7.87 ± 2.2	87
301–350	67.53	5	7.96 ± 2.1	86
351–400	68.87	4	7.64 ± 2.5	77
OWe				
150–200	54.87	10	0.278 ± 2.1	73
201–250	54.89	15	0.276 ± 1.5	74
251–300	55.62	15	0.266 ± 1.3	70
301–350	56.21	5	0.256 ± 1.2	71
351–400	56.99	4	0.276 ± 1.5	73
<i>Lethrinus letnjan</i>				
OL				
160–210	53.78	12	11.1 ± 1.6	56
211–250	53.89	11	10.9 ± 1.7	59
251–300	54.11	11	10.2 ± 1.2	61
301–350	54.78	13	10.9 ± 1.5	62
351–400	55.61	10	10.6 ± 1.3	73
401–450	55.81	9	11.2 ± 1.1	74
451–500	56.43	6	10.5 ± 1.3	72
OW				
160–210	77.35	12	7.59 ± 1.3	81
211–250	77.69	11	7.49 ± 1.1	80
251–300	78.65	11	7.39 ± 1.3	83
301–350	78.89	13	7.54 ± 1.2	77
351–400	79.21	10	7.64 ± 2.1	79
401–450	79.75	9	7.72 ± 2.5	82
451–500	79.99	6	7.62 ± 2.4	80

(Continued)

Table 2

Continued

Character	CV ² _a	N	Character mean (mm) ± SD	% of individuals with asymmetry
OWe				
160–210	51.94	12	0.328 ± 1.3	66
211–250	52.22	11	0.321 ± 1.1	62
251–300	52.87	11	0.319 ± 1.4	69
301–350	53.55	13	0.324 ± 2.1	77
351–400	53.87	10	0.325 ± 2.4	72
401–450	54.44	9	0.322 ± 2.3	78
451–500	54.97	6	0.321 ± 2.1	79
<i>Lethrinus mahsena</i>				
OL				
160–210	30.87	9	7.4 ± 1.4	70
211–250	31.33	8	7.7 ± 1.1	72
251–300	31.87	7	7.5 ± 2.3	78
301–350	32.55	5	8.1 ± 2.1	79
351–400	32.89	5	7.8 ± 1.5	82
401–450	32.99	7	7.4 ± 1.1	84
451–500	33.76	7	7.8 ± 1.2	87
501–550	33.87	6	7.7 ± 1.1	89
OW				
160–210	76.64	9	6.1 ± 1.1	78
211–250	76.99	8	6.3 ± 1.4	79
251–300	77.22	7	6.4 ± 1.1	81
301–350	77.83	5	6.1 ± 1.5	82
351–400	77.99	5	6.2 ± 2.1	84
401–450	78.21	7	6.4 ± 1.1	87
451–500	78.65	7	6.3 ± 1.4	88
501–550	78.99	6	6.4 ± 1.1	89
OWe				
160–210	50.85	9	0.227 ± 1.3	83
211–250	50.99	8	0.229 ± 1.1	85
251–300	51.33	7	0.228 ± 2.1	87
301–350	51.78	5	0.230 ± 1.4	81
351–400	52.44	5	0.229 ± 1.1	89
401–450	52.65	7	0.227 ± 1.4	90
451–500	53.99	7	0.230 ± 1.4	83
501–550	54.43	6	0.229 ± 1.1	87

OL, otolith length; OW, otolith width; OWe, otolith weight; SD, standard deviation.

the three lethrinid fish inspected is the OWe. The asymmetry found in this study between the left and right sides of the fish head has previously been quantified for explicit for which information is available



(Al-Rasady et al., 2010; Abu El-Regal et al., 2016), which could maintain the vulnerability of the OWe to instant disparities in the habitat. Accordingly, this fundamental modification in the OWe approximation might be contemplated an actual bioindicator of strain in the Southern Red Sea territory. Moreover, the OL revealed the lowest bilateral asymmetry stage in all three lethrinid species immediate. It proposes that this characteristic could not be used as a biomarker to evaluate pollution. Then, this smaller asymmetry amount might be explained, as the developing stage of the OL and could not be linked to non-favourable ecological environments (Jawad, 2003).

Earlier investigations on otoliths discovered that numerous benthic and pelagic species with a mean level of OWe asymmetry fluctuating from 0.2 to 0.2 (Jawad, 2013; Jawad & Sadighzadeh, 2013; Lychakov et al., 2008). In this study, a higher weight asymmetry value for the three lethrinid species corresponds with the findings of Bouriga et al. (2021) on *Trachurus mediterraneus* collected from various localities in the Tunisian waters of the Mediterranean Sea. Bouriga et al. (2021) related such increased levels in the asymmetry of the size of the otolith to the biological state of these species, their places, and natural pressure (abiotic and biotic), where habitat and human caused impacts have a notable effect on the development of otoliths (Grønkaer, 2016). The present finding disagrees with those of Reis et al. (2023) on four mugilid species examined from Köyceğiz Lagoon, Aegean Sea, Turkey. They explored the level of discrepancies in the otolith of four mullet species *Chelon auratus*, *C. labrosus*, *C. saliens*, and *Mugil cephalus*. In addition, they found that the irregularity grade of OL was greater than that of OW and weight. They suggested that the likely reason of irregularity in the otolith traits inspected could be the inconsistency in growing provoked by habitat influence associated with the discrepancy in water temperature, salinity, depth, and impurities present in the Köyceğiz Lagoon body.

In assessment with the outcomes reached in this study, analogous and different conclusions have been accomplished by many investigators for numerous fish species worldwide. For example, Mejri et al. (2020) assessed unevenness in the otolith shape, length, width, and area in *Pagellus erythrinus* collected from the Gulf of Tunis and found intra- and inter-population asymmetry in these characteristics and considered this irregularity in the view of the rising inconsistency provoked by genetic and environment impacts. Jawad (2012) discovered that the level of asymmetry in the OW is at its highest value for the length and width data attained for *Lutjanus bengalensis* collected from the Muscat coast on the Sea of Oman, with a propensity to upsurge irregularity in the length and width as the

fish increases in length. Moreover, this asymmetry has been attributed to the several pollutants present in the region. Additionally, Jawad et al. (2012a, 2012b) in *Sardinella sindensis* and *Sillago sihama* collected from the Arabian Gulf near Bandar Abbas and Jawad et al. (2020) in *Sarotherodon melanotheron* Rüppell, 1852, and *Coptodon guineensis* studied from Lake Ahémé and Porto-Novo Lagoon, Bénin, achieved comparable outcomes, but an increase in the values of asymmetry in the OW only. Yet, Al-Busaidi et al. (2017) affirmed that the OL and OW sizes assessed from *Lutjanus ehrenbergii* in the Sea of Oman at Muscat City are suitable symbols for fish length, and there is regularity between the left and right otoliths. Furthermore, Kondaş et al. (2019) inspected the fluctuating asymmetry of the otolith area, length, perimeter, and width in four groups of *Merlangius merlangus* collected from the Middle Black Sea. They discovered that the otolith area had the topmost irregularity, where the length had the lowest. Also, they recommended no substantial association between the asymmetry amounts of the four otolith traits and the total length. The unevenness in these traits could be an outcome of the influence from varied pollutants in the Black Sea. Besides, Chakour and Elouizgani (2018) stated noteworthy asymmetry between the OL and perimeter. Changes in width in three groups of *Solea lascaris*, taken in three major seaports of the Moroccan Central Atlantic coast showed that the changes between these traits can be due to the human-induced causes and the adaptability of each group to its locality. Also, the outcomes of Abu El-Regal et al. (2016) presented an asymmetry in the length and width of the otoliths in *Chlorurus sordidus* and *Hipposcarus harid* examined from Hurghada, Red Sea coast of Egypt. They revealed a gain of asymmetry as the fish length (age) becomes more substantial, and such an upsurge in the asymmetry value might be attributed to the occurrence of pollutants in the region. On the other hand, Fey et al. (2022) studied the fluctuating asymmetry in the fry of *Esox lucius* to determine whether skeletal and body deformities of northern pike fry (3–12 cm standard length [SL]) reared under laboratory conditions imposed stress that was reflected in fluctuating asymmetry in otolith size (area). Although hatchery-reared fish were studied, fluctuating asymmetry also occurs in natural conditions and may indicate harmful effects of different environmental factors, including water pollution. Fey et al. (2022) used both the sagitta and lapillus in their analyses on fish with ($n = 60$) and without ($n = 60$) skeletal deformities. They showed that despite the severity of deformity and extended duration of stress exposure (from hatching to ca.

2–3 months of life), there was no significant effect of the stressor on otolith fluctuating asymmetry, neither for sagittae nor lapilli. Fey et al. (2022) concluded that an overview of the literature indicated that links between stressors and fish otolith fluctuating asymmetry are not common (found in 9 out of 31 investigations). Consequently, otolith fluctuating asymmetry is probably not an appropriate bioindicator for detecting the effects of different stressors, including pollution, despite previous suggestions.

Numerous investigators have anticipated that genetic issues could be responsible for the unevenness on both sides of the otolith (Panfili et al., 2005). However, this issue cannot be deliberated here due to the lack of genetic information on the three lethriniid species examined. Finally, the present outcome adds to the data on otolith shape and morphometry information that are perfect gears for discriminating and remarking the irregularity in otolith features from both sides of fish of the lethriniid species selected for this investigation. The irregularity observed here in the OWe can be associated with the habitat matters, as with water temperature, salinity, and impurities. However, this research suggests further studies to investigate asymmetry in otolith features such as the shape factor, circularity, ellipticity, and rectangularity, as well as genetic investigates that are essential to acknowledge the causes that led to asymmetry in the otolith pairs of the three lethriniid species examined at the present time in the southern part of the Red Sea.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data underlying this article will be shared upon reasonable request to the corresponding author.

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