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Investigation of otolith asymmetry in *Mulloidichthys flavolineatus* and *Parupeneus forsskali* (Perciformes: Mullidae) from Egypt's Hurghada fishing harbour on the Red Sea

by

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Abstract

The current study aims to calculate and assess the asymmetry of the two goatfish species, Yellowstripe goatfish Mulloidichthys flavolineatus (Lacepède 1801) and Red Sea goatfish, Parupeneus forsskali (Fourmanoir & Guézé 1976) collected from Hurghada fishing harbour, Egypt. The asymmetry valuation for M. flavolineatus and P. forsskali is imperative to demonstrate the impact of asymmetry on the larvae settlement in this vital fishing ground. Asymmetry was calculated for the saccular otolith (Sagittae) biometry, namely length, width, and mass. The results showed that the otolith height had a lower asymmetry value than the otolith length for the two goatfish species inspected. No relationship between the asymmetry value of otolith length and width and total fish length was observed. Both goatfish species' calculated otolith mass asymmetry was higher than that of many teleost fish species.

Key words: otolith length, otolith height, otolith mass, measurements, morphometrics

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1. Introduction

Mulloidichthys flavolineatus is a marine species associated with reefs at a depth range of 1-76 m (Allen & Erdmann 2012). It reaches a maximum total length of 430 mm (Lieske & Myers 1994), and a maximum reported weight of 423.30 g (Mehanna et al. 2017). Individuals of this species occasionally form schooling that inhabits shallow sandy areas of lagoons and seaward reefs (Lieske & Myers 1994). The large adults of this species frequently live solitary on sand slopes with other species. This species feeds on crustaceans, molluscs, worms, heart urchins, and foraminifera. It is distributed in the Indo-Pacific region as well as in the Arabian Gulf (Wright 1988), the Red Sea, and East Africa to the Hawaiian, Marguesan, and Ducie islands, north to the Ryukyu and Bonin Islands, and south to Lord Howe and Rapa Islands (Lieske & Myers 1994).

Parupeneus forsskali is a marine species that sometimes enter brackish water. It is associated with reefs at a depth range of 1–45 m (Sonin et al. 2013). It reaches a maximum total length of 280 mm (Burgess et al. 1999) and a maximum recorded weight of 275.30 g (Mehanna et al. 2017). This species is the most common goatfish in shallow water in the Red Sea. Individuals of this species live on sand bottoms near coral reefs (Kumaran & Randall 1984). They feed on invertebrates that live on sand (Hobson 1974). It is distributed in the Indian Ocean region, the Red Sea, Gulf of Aden, and Socotra (Randall 2004). Moreover, it was reported from the Suez Canal and migrated to the Mediterranean Sea, where it established itself (Froese and Pauly 2022).

The otoliths of the teleost fishes are hard and solidified structures, usually found in the fish's inner ear. Anatomically, there are three pairs of otoliths in the inner ear of fish consisting mainly of calcium and carbonate contents precipitated in an organic matrix (Marmo 1982).

Fluctuating asymmetry refers to slight differences in the symmetry of small-paired structures, including otoliths, in bilaterally symmetrical animals (Van Vallen 1962). Such differences result from an inability to control development under environmental or genetic stress (Jawad 2001). High degrees of fluctuating asymmetry in otoliths may suggest high levels of environmental stress associated with degraded habitats (Jawad et al. 2012).

Relationships between fish health status and fluctuating asymmetry have been explored for several anatomical structures in fish. These include the number of gill rakers, pectoral fin rays, fish body proportions, eyespot area, and otolith size and shape (Jawad 2001, 2003, 2004; Jawad et al. 2012; Ben Labidi et al. 2021; Ben Labidi et al. 2020a,b). Measurement of the fluctuating asymmetry degree in the otoliths would be helpful for fisheries managers as it can provide an index of past individual and population suitability over time if time series of collections are available. Such data may inform forthcoming management policies (Jawad et al. 2012).

The coefficient of otolith mass asymmetry has been utilised as a bioindicator to test the health status of various aquatic environments (Grønkjær 2016) and to test diverse environmental impacts on fish populations.

The extent of asymmetry in otolith dimensions and weight has been investigated in several fish species across a range of diverse environments from different countries. These include Acanthopagrus bifasciatus and A. latus from Iragi marine waters (Abdulsamad et al. 2020), Beryx splendens from the Arabian Sea coast of Oman (Albusaidi et al. 2010), Liza Kluzingeri from the Persian Gulf (Sadigzadeh et al. 2011), Carangoides caeruleopinnatus from Oman Gulf (Jawad et al. 2012c), Merlangius merlangus from the Black Sea (Kontaş et al. 2018) and Engraulis australis from the Hauraki Gulf, New Zealand (Ben Labidi et al. 2020b), Boops boops, (Bouriga et al. 2021), six benthic and pelagic fish species from Gulf of Tunis, (Khedher et al. 2021), Diplodus vulgaris (Mejri et al.), 2022a Pagellus erythrinus (Mejri et al. 2022b), Liza aurata and Chelon ramada (Jawad & Adams 2021). In the Red Sea area, the asymmetry in the otolith dimensions was studied in Chlorurus sordidus and Hipposcarus harid (El Regal et al. 2016). Jawad et al. (2017) assessed the otolith mass asymmetry in Chlorurus sordidus and Hipposcarus harid.

The present study aimed to calculate and assess the asymmetry of the otolith length, width, and weight of the two goatfish species, Yellowstripe goatfish *Mulloidichthys flavolineatus* (Lacepède 1801) and Red Sea goatfish, *Parupeneus forsskali* (Fourmanoir & Guézé 1976) collected from Hurghada fishing harbour, Egypt. The asymmetry valuation for *M. flavolineatus* and *P. forsskali* is imperative to demonstrate the impact of asymmetry on the larvae settlement in this vital fishing ground.

2. Materials and methods

Hurghada is in the northern part of the Red Sea between latitudes 27° 10′ N – 27° 33′ N and longitudes 33° 70′ E – 33° 85′ E. It is located 500 km southeast of Cairo on the western coast of the Red Sea. It extends for around 36 kilometres along the seashore but does not reach the nearby desert. Hurghada is adjacent to an important fishing area for the two goatfish species assessed in the present study.

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A total of adult 158 of M. flavolineatus and 102 of P. forsskali specimens (Fig. 1) were obtained monthly from the commercial catches at the Hurghada landing site (27° 15′ 46″ N 33° 49′ 17″ E) (Fig. 2) during 2021. After dissection, sexual maturity of both species individuals was determined by examining the gonads using a light microscope. Sexes were not separated for this study and left for a future investigation on the bilateral asymmetry and its relation to the sex of the fish. Gill nets and trammel nets were used to catch the fish. The length of the nets varied between 10 and 40 m long with a mesh size of 0.5 – 3 mm. The total length 'TL' of fish (to the nearest 0.1 cm) was measured from the anterior border of the head to the posterior of the caudal fins using a digital caliper. Total body weight 'W' grams (to the nearest 0.01 g) were measured. After dissection, sex was determined by examining the gonads using a light microscope. The right and left sagittal otoliths were extracted through a cut in the cranium to uncover them, then washed and kept dry in glass vials. It is worth to mention that this study did not include an otolith sample with a clear sign of calcite crystallisation, or other abnormal features as positioned with the sulcus acusticus oriented towards the observer. The otolith length was



Figure 1

(A) *Mulloidichthys flavolineatus* 255 mm TL collected from the Red Sea coast, Egypt; (B) *Parupeneus forsskali* 250 mm TL collected from the Red Sea coast, Egypt.



Map showing sampling locality at Hurghada at the northern part of the Red Sea, Egypt

measured using an ordinary light microscope. Both the maximum otolith length (OL) (the longest-most horizontal length across the otolith) and the maximum otolith height (OH) (the longest vertical length across the otolith) were measured to the nearest 0.01 mm, recording the greatest distance from the anterior tip to the posterior edge (OL) and the greatest distance between the otolith dorsal and ventral margins (OH) (Fig. 3), after (Harvey et al. 2000) and Battaglia et al. (2010). The saccular otolith mass asymmetry was determined as the discrepancy between the weight of the right and left otoliths divided by the mean otolith mass in the two goatfish species examined. The value of asymmetry (X) in general, which include all the biometric measurements plus maximum otolith length and maximum otolith height asymmetry, was calculated using the following formula given by Ben Labidi et al. (202a):

$$X = (M_R - M_L) M_M^{-1}$$

where M_{R} and M_{L} are the otolith weights of the right and left otolith pairs, and M_{M} is the mean weight of the right and left otolith pairs.

The X values vary from -2 to +2. These limit values indicate maximal asymmetry while the '0' value denotes that there is no asymmetry between right and left otoliths. A positive value of X means that the left otolith is lighter than the right one ($M_1 < M_p$), while a

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Otolith of (A) Mulloidichthys flavolineatus 255 mm TL; (B) Parupeneus forsskali 250 mm TL collected from the Red Sea coast, Egypt

negative value of X indicates that the left otolith is heavier than the right one $(M_1 > M_p)$.

Statistical calculations were built on the squared coefficient of dissimilarity variation (CV_a^2) for the length, width, and weight of the otolith in accordance with Valentine et al. (1973):

$$CV_a^2 = \left(\frac{S_{r-l}X100}{X_{r+l}}\right)^2$$

where:

 S_{r-1} = the standard deviation of signed variances,

 X_{r+1} = the mean of the feature that is measured by adding the total counts for two sides of the fish head and dividing by the number of fish specimens investigated.

Individual errors in measuring sizes can mask the results of dissimilarity calculations that make it useless (Palmer 1994). Thus, to avoid any unsolicited fault in the present study, the sizes were recorded by a single reader and achieved two times (Lee & Lysak 1990). The average measurements were used in this analysis. Dissimilarity coefficients were assessed between the various length groups using the ANOVA test. Fish specimens of both species in question were separated into groups according to their total length and the asymmetry coefficient was calculated for the biological features of the otolith of each length group. Accordingly, five and four total length groups were identified in *M. flavolineatus* and *P. forsskali* respectively.

3. Results

The examination of asymmetry data of the otolith length, width and weight of M. flavolineatus and P. forsskali collected from Hurghada on the Red Sea coast of Egypt are given in Table 1. The sagittae from 158 specimens (101–350 mm TL) of M. flavolineatus and 105 specimens (101-300 mm TL) of P. forsskali were collected. The value of the asymmetry coefficient of the biological parameters of the otolith of M. flavolineatus and P. forsskali were obtained as a result of applying the formula of CV_a^2 given in the materials and methods section mentioned above. For M. flavolineatus, the value of the asymmetry of otolith length is 23.37, the value of the asymmetry of the otolith height is 23.01, and the value of the otolith mass asymmetry is 0.2413 (Table 1). For P. forsskali, the value of the asymmetry of the otolith length is 33.74, the value of the asymmetry of the otolith height is 34.81 and the value of the otolith mass asymmetry is 0.305 (Table 1).

Table 1

The squared coefficient of asymmetry (CV_a²) value and character mean (otolith length, width in mm, and mass in g) values of *Mulloidichthys flavolineatus* and *Parupeneus forsskali* were examined in the present study. N, the total number of otolith retrieved from 158 fish individuals for *Mulloidichthys flavolineatus* and 102 individuals for *Parupeneus forsskali*.

Character	CV _a ²	N	Character means	% of individuals with asymmetry			
Species							
Mulloidichthys flavolineatus							
Otolith length	23.37	316	3.61 ± 0.43	96			
Otolith height	23.01	316	2.59 ± 0.35	92			
Otolith mass	0.2413	316	0.0045 ± 0.11	66			
Parupeneus forsskali							
Otolith length	33.74	204	3.30 ± 0.22	90			
Otolith height	34.81	204	2.11 ± 0.16	93			
Otolith mass	0.305	204	0.0031 ± 0.21	64			

The otolith mass asymmetry value is the largest asymmetry level among the three otolith variables studied in this species. For *M. flavolineatus*, ninety-six percent of the individuals showed asymmetry. This is a higher percentage level obtained for the three otolith parameters (Table 1).

A trend of increase in the asymmetry values was noticed in the three biological features of the otolith of both species in question with the increase of the fish total length.

4. Discussion

This work was done to express the bilateral asymmetry in otolith characteristics such as length, width, and weight. The observed asymmetry in both the length and width of the otolith of *M. flavolineatus* and P. forsskali may cause a reduction in the ability of the young individuals to settle down and be found in their appropriate environments (Gagliano et al. 2008). Such a defect will lead to the uncertainty of knowing their home waters by the young individuals of the fish species. With respect to commercial fishing, such a problem will end up causing a deficit in the stock of a certain species. Since M. flavolineatus and P. forsskali are among the commercial species, the effect of asymmetry on the otolith of the young of these two species will absolutely affect the stock of these species in the area they inhabit. During dispersal, individuals are faced with the major challenge of constantly gathering information on the variable conditions of the surrounding environment in order to survive (Cote & Clobert 2007). In coral reef fishes, the question of how centimetre-sized larvae orient themselves towards coral reefs following weeks of dispersal at sea remains one of the biggest unsolved questions in marine science. However, the smell and sound of coral reefs have recently been demonstrated to be significant navigation cues for the successful recruitment of reef fishes to benthic habitats (Gerlach et al. 2007).

Reef sound has emerged as a leading candidate mechanism because acoustic signals can propagate in water over many kilometres and with little attenuation (Rogers & Cox 1988). Although experimental evidence of the hearing abilities of reef fish larvae is only recent and limited, we now know that pelagic reef fish larvae home in on reef-associated sounds to actively locate and move towards their juvenile and adult benthic reef habitats (Tolimieri et al. 2004; Wright et al. 2005). Yet given that coral reefs are particularly noisy environments (Cato 1978), a suite of sound sources of mixed frequencies and intensities may easily obscure the detection and selection of the appropriate settlement habitats. By being capable of tuning into a specific frequency noise, pre-settlement larval fishes may get a better indication of the biological and physical states of the habitat they are approaching. Indeed, it has recently been demonstrated that settlement-stage larvae can actively discriminate between different components of reef sound, and some preferentially settle onto reefs that produce high-frequency noise above 570 Hz (Simpson et al. 2005).

The integrity of the saccule, the primary auditory organ in fishes, has a direct bearing on what and how fish larvae hear. Saccular otoliths greatly affect individual's ability to effectively detect sound (Popper & Lu 2000). Fishes depend on a comparison of left versus right saccular input to the brain to resolve the azimuth of the sound direction (i.e., inter-saccular analyses; Edds-Walton & Fay 2002), and morphological differences between the right and left paired saccular otoliths can pose problems for sound localisation due to incongruity in the movement of the right and left otolith within the saccule (Lychakov & Rebane 2005). Hence, even a small degree of asymmetry in the shape of the otolith pair can cause significant differences in the acoustic functionality of young fishes, thereby potentially jeopardising the successful recruitment of larvae to suitable benthic habitats.

The morphological characteristics of the fish are usually susceptible to changes in environmental Therefore, factors. the extreme asymmetry levels of the otolith length may indicate such an effect. The information on the association among numerous environmental contaminations and the morphometrics of the two red mullet species examined in the current work need to be included, which in turn makes it impossible to evaluate asymmetry alongside a pollution gradient. Hence, it is not plausible at this stage to have an accurate suggestion of the implication of this phenomenon. In the present study, M. flavolineatus and P. forsskali were investigated from only one locality (Hurghada fishing harbour). At the same time, no prior data exists on its asymmetry fluctuating to allow decisions and appraise the fluctuating asymmetry level. However, the present findings on the otolith size asymmetry in M. flavolineatus and P. forsskali will be considered a case study for future studies and a reference record for comparison.

Previous investigations on otolith revealed several benthic and pelagic species with a mean value of otolith mass asymmetry from -0.2 to 0.2 (Lychakov et al. 2008; Jawad 2013; Jawad & Sadighzadeh 2013). In the study, a higher mass asymmetry value for *M. flavolineatus* (0.2413) and *P. forsskali* (0.3045) was

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attained. This result agrees with that of Bouriga et al. (2021) on Trachurus mediterraneaus collected from different sites on the Tunisian coasts of the Mediterranean Sea. Bouriga et al. (2021) related such high values to the physiological condition of these species, their habitat, and environmental influences (abiotic and biotic), where ecological and anthropogenic pressures have a remarkable effect on the growth of otoliths (Grønkjær 2016). The present results are also in agreement with those of Ben Labidi et al. (2020b). The results of Ben Labidi et al. (2020b) showed that the highest level of asymmetry between the two populations of the sparid species Boops boops collected from Bizerte and Kelibia localities along the Tunisian coasts was in the otolith area followed by otolith length and otolith height. Nevertheless, a significant asymmetry was found only in the otolith height. Ben Labidi et al. (2020b) suggested that the asymmetry attained between the right and left otoliths within the Kelibia population has earlier been reported in some fish species for which data are available, which could hold the vulnerability of the otolith height to instant variations in the environment. Ben Labidi et al. (2020a) added that the significant difference in the otolith height value attained can be used as a definite biomarker of stress in the Kelibia environment. In the case of the otolith length, the present results also agree with those of Ben Labidi et al. (2020a) in having an asymmetry in this biological feature of the otolith of M. flavolineatus and P. forsskali examined. The level of asymmetry of the otolith length obtained by Ben Labidi et al. (2020a) was low, and they explained such low value because the developmental period of the otolith length may not be consistent with the presence of adverse environmental events (Jawad 2003). As previously mentioned by Helling et al. (2003), the ostensible impact of bilateral asymmetry in fish otoliths is abnormal swimming activity and intrusion with correct sound localisation, resulting in the inability of the individuals to integrate with the environment in which they live (Lychakov & Rebane, 2005). As with the results attained in the present study, Mejri et al. (2020) examined asymmetry 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 characters, except for the surface, and discussed this fluctuating asymmetry given the developmental variability affected by genetic and environmental impact. Jawad (2012) mentioned that the level of asymmetry in the otolith height is the highest among the length and width values of Lutjanus bengalensis collected from the Muscat coast on the sea of Oman, with a trend towards increasing asymmetry in the length

and width as the fish increases in length, adding that this asymmetry is attributed to the various pollutants present in the area. Similarly, Jawad (2012) and Jawad et al. (2012a) in Sardinella sindensis and Sillago sihama collected from the Persian Gulf near Bandar Abbas and Jawad et al. (2020) in Sarotherodon melanotheron and Coptodon quineensis examined from Lake Ahémé and Porto-Novo Lagoon, Bénin, found the same results. Al-Busaidi et al. (2017) stated that both the length and width measurements of the otolith estimated from Lutjanus ehrenbergii in the Sea of Oman at Muscat City are good indicators for fish length, and there is symmetry between the left and right otoliths. Furthermore, Abu El-Regal et al. (2016) found an asymmetry in the length and width of the otolith in Chlorurus sordidus and Hipposcarus harid examined from Hurghada on the Red Sea coast of Egypt and detected an increase of asymmetry as the fish length (age) increased and attributed the possible cause of the asymmetry in the two species to the presence of pollutants in the area.

Environmental impacts on fish otolith growth are not an unnoticed process as both natural environmental challenges and anthropogenic challenges have reported such effects (Grønkjær 2016). Different studies attributed the variability of otolith morphology to the impact of the environment; we can quote: Trojette et al. (2015), who reported the otolith shape variation for the two insular populations of *Diplodus annularis* (Linnaeus 1758) along the Tunisian coast to the environmental factors. Mejri et al. (2018) also gave the same suggestions in their work on *Pagellus erythrinus* in Tunisian waters.

Pollutants severely influenced Egypt's Red Sea coast (Mansour et al. 2013; Perry et al. 2015; Youssef et al. 2020). Mansour et al. (2013) suggested that several anthropogenic and natural disturbances influence the marine environment of the Hurghada area including pollution by: oil spills, wastewater discharge, effluents of desalination plants, and building activities along the seashore, marine traffic, and landfilling and dredging operations. In addition, several studies on marine surface sediments of the Egyptian Red Sea have been carried out (Madkour et al. 2012). The marine environment of the Hurghada coastal area has suffered substantial environmental impact from anthropogenic activities due to the increasing population growth and the rapid development taking place in the tourism industry and their negative impacts on the marine environment (Mansour et al. 2013). The different human activities performed in Hurghada can adversely affect the marine coast and marine ecosystem. Many studies have assessed heavy metal concentrations along the Red Sea coast (Abdelkader et al. 2018; Nour et al. 2018, 2019). The high metal concentrations of Youssef et al. (2015) showed that the high concentration of Pb and Cd is due to anthropogenic sources (mean 38.76 and 2.43 mg g⁻¹ respectively) and these are essentially attributable to the anomalous concentrations of Pb and Cd in the results of Youssef et al. (2015).

Table 2

The squared coefficient of asymmetry (CV_a²) and character means (otolith length, width in mm, and mass in g) by the fish total length of *Mulloidichthys flavolineatus* and *Parupeneus forsskali* examined in the present study. N, number of otoliths obtained from specimens of each total length group.

Character	CV _a ²	N	Character mean	% Of individuals with asymmetry			
Mulloidichthys flavolineatus							
Otolith length							
101 – 150	23.25	16	3.60 ± 0.32	75			
151 – 200	23.43	152	3.59 ± 0.14	99			
201 – 250	23.74	116	3.58 ± 0.21	93			
251 – 300	23.95	24	3.49 ± 0.75	100			
301 – 350	23.98	8	3.57 ± 0.31	100			
Otolith height							
101 – 150	21.15	16	3.57 ± 0.11	63			
151 – 200	21.46	152	3.49 ± 0.16	96			
201 – 250	21.79	116	3.59 ± 0.15	90			
251 – 300	21.85	24	3.52 ± 0.23	100			
301 – 350	21.99	8	3.55 ± 0.22	100			
Otolith mass							
101 – 150	0.2410	16	0.0045 ± 0.25	50			
151 – 200	0.2418	152	0.0043 ± 0.40	74			
201 – 250	0.2425	116	0.0041 ± 0.27	64			
251 – 300	0.2432	24	0.0038 ± 0.42	25			
301 – 350	0.2487	8	0.0037 ± 0.33	100			
Parupeneus forsskali							
Otolith length							
101 – 150	33.72	16	3.28 ± 0.14	50			
151 – 200	33.85	138	3.29 ± 0.13	94			
201 – 250	33.87	44	3.27 ± 0.22	92			
251 – 300	33.99	6	3.26 ± 0.23	100			
Otolith height							
101 – 150	32.79	16	2.10 ± 0.11	63			
151 – 200	32.85	138	2.09 ± 0.19	94			
201 – 250	32.93	44	2.07 ± 0.18	96			
251 – 300	32.98	6	2.08 ± 0.13	100			
Otolith mass							
101 – 150	0.3041	16	0.0031 ± 0.02	38			
151 – 200	0.3047	138	0.0030 ± 0.08	65			
201 – 250	0.3068	44	0.0029 ± 0.06	64			
251 - 300	0 3086	6	0 0028 + 0 05	100			

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As verified in previous studies (Al-Mamry et al. 2011a, 2011b; Jawad et al. 2012a, 2012b, 2012c; Mabrouk et al. 2014; Jawad and Adams 2021), the results of this study showed that large-size specimens of M. flavolineatus and P. forsskali have higher dissymmetry value than younger individuals. It was clear that the extent of fluctuating asymmetry of the length and width and the mass of the otolith increased with the fish size (Table 2). Comparable results were gained by Valentine et al. (1973) in chosen fish species collected from California, U.S.A. They suggested two likely theories that might account for such an approach: ontogenetic disparities related to a surge in dissymmetry with size (age), and possible historical occasions which resulted in an increase in dissymmetry. Otherwise, Thiam (2004) mentioned that a growing propensity in dissymmetry value with fish size could be because of the exposure of grown-up species to hostile environment for a longer period.

Otolith shape asymmetry and otolith mass has been poorly examined in fishes of this part of the world, unlike in other parts of the world. So far, on the Egyptian coast of the Red Sea, the study of the otolith mass asymmetry was obtained only for *Chlorurus sordidus* and *Hipposcarus harid* (El-Regal et al. 2016). Hence, more research investigating the effect of the environment on otolith mass asymmetry and on fish behaviour is needed. Accordingly, it is vital to comprise many specimens and a wide range of body sizes to fully distinguish the relationship between the asymmetry in otolith mass and the fish length.

Ethical statement

This work is based on commercial fish species, and the specimens were collected from a commercial catch. Therefore, ethical aspects are not applicable.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions L.A.J. developed the research idea and wrote the manuscript with input from all authors; Y. A. M. collected the samples and partially analysed data; A.L.I. analysed data and drew graphs; A. M. Q., M. F., A. O., S. F. M., and M. S.-K. contributed to the data analysis and to writing the manuscript.

Data availability statement

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

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