COMPARATIVE STUDY OF OTOLITH BILATERAL ASYMMETRY BETWEEN TWO FISH SPECIES OF THE FAMILY MULLIDAE: THE NATIVE *MULLUS BARBATUS* AND THE LESSEPSIAN MIGRANT *UPENEUS PORI*

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Abstract

Otolith bilateral asymmetry may reflect the environmental stress experienced by fish during their life. The present study compared the otolith asymmetry between two species of the family Mullidae, the native *Mullus barbatus* (red mullet) and the alien *Upeneus pori*, in order to evaluate the degree of adaptation of *U. pori* to its new environment. The analysis of 10 otolith traits (size and shape descriptors) showed that otolith asymmetry was not higher in the alien species which suggests that *U. pori* is comparatively well adapted to its new habitat. Future research is warranted in order to further assess the utility of otolith bilateral asymmetry as a bioindicator for evaluating the degree of adaptation of Lessepsian migrants.

Keywords: otolith morphology, alien species, adaptation.

1. Introduction

Otolith morphology is determined by genetic and environmental interactions and presents a sensitive indicator of developmental errors (Geladakis *et al*., 2021, 2022). In particular, otolith bilateral asymmetry is a popular tool for examining the effects of environmental stress on the health status of wild populations (Gagliano *et al*., 2008). The aim of the present study was to explore the use of otolith asymmetry as a bioindicator for evaluating the adaptation of invasive species to their new (foreign) habitats. For this purpose, a comparatively study was performed on two species of the family Mullidae, namely, the native *Mullus barbatus* (red mullet) and the alien *Upeneus pori*, caught in the Gulf of Heraklion Crete.

U. pori is a subtropical species, distributed along the western Indian Ocean (Ben-Tuvia & Golani, 1989). It entered the Mediterranean Sea via the Suez Canal (Lessepsian migrant) and was first recorded in 1950 (Deidun *et al*., 2018). Since its initial detection, *U. pori* has expanded its distribution in the Mediterranean Sea and established successful populations (Deidun *et al*., 2018).

2. Material and Methods

A total of 105 and 117 specimens of the native (*M. barbatus*) and alien (*U. pori*) species were used for this study. All individuals were caught with an experimental bottom trawl (with 40-mm stretched, diamond-mesh net in the cod-end) on board the R/V PHILIA during the “*LeFish*” research action (supported by “*RePhil*”) in the Gulf of Heraklion, Crete, on January 20-21, 2022. Trawling was carried out on a shallow (30-35 m) sandy area covered with the seaweed *Caulerpa prolifera*.

In the laboratory, each fish was measured for standard length (SL, tip of snout to base of the central caudal lepidotrichia, mm) and weighed (TW, g). The length-weight relationship ($TW = a \times SL^b$) was fitted in both species by linear regression analysis after logarithmic transformation of TW and SL. From each specimen, the largest pair of otoliths (*sagittae*) was removed and individually photographed. All otolith images were analyzed using the “ShapeR” package (Libungan & Palsson, 2015), open software in R (version 4.0.03, R Core Team, 2020). After the extraction of otolith contours, otolith bilateral asymmetry was assessed for ten traits; four univariate morphometric descriptors (maximum length, O_L; maximum depth, O_D; surface, O_S; perimeter, O_P) and six high-amplitude harmonics (H_2-H_7), produced by a normalized elliptic Fourier technique (Geladakis *et al.*, 2021, 2022).

All traits were tested for the type of bilateral asymmetry, i.e., fluctuating asymmetry, directional asymmetry or antisymmetry (Somarakis *et al.*, 1997). Specifically, one-sample t-tests were carried out to evaluate the presence of directional asymmetry (skew, g1≠0) and antisymmetry (kurtosis, g2≠0) in the R-L (right minus left) distribution for each otolith trait. The variance of the bilateral difference [index FA_1=var(R-L)] was estimated for each species and otolith trait, as an index of fluctuating asymmetry (Palmer & Strobeck, 1986). The significance of differences in FA_1 between the two species was tested using F-tests (Somarakis *et al.*, 1997).

3. Results

The standard length (SL) of the native fish (*M. barbatus*) ranged from 69 to 104 mm, whereas of the alien fish (*U. pori*), from 76 to 127 mm. The growth in weight of *M. barbatus* presented a uniform allometric pattern (Fig. 1A). Instead, in the case of *U. pori*, the TW-SL relationship presented an inflection point at 85.9 mm SL (logSL=1.935), above which the rate of TW increase was lower (blue points in Fig. 1B).

For all otolith traits examined, antisymmetry was absent, with the kurtosis (g2) estimates being either non-statistically different from zero or significantly positive (leptokurtic). Directional asymmetry was detected for the shape descriptors H_4 and H_7 (skew significantly positive, R > L) and the size descriptors O_D and O_P (skew significantly negative, R < L). However, fluctuating asymmetry indices based on signed R–L values (e.g., FA_1) are not biased by skewed distributions (Palmer & Strobeck, 1986). Interestingly, for four shape morphometric descriptors (H_2, H_3, H_4, H_6), FA_1 was significantly higher in the native *M. barbatus* compared to the alien *U. pori* (Fig. 2).

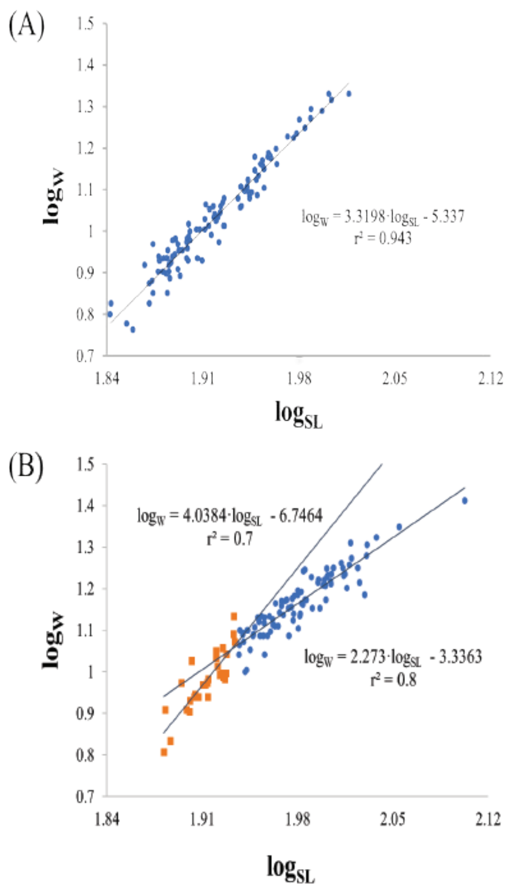

Fig. 1: Allometric relationship between total weight (TW, g) and standard length (SL, mm). (A) *Mullus barbatus* and (B) *Upeneus pori.* Different colors in *U. porri* indicate the change of the TW-SL relationship.

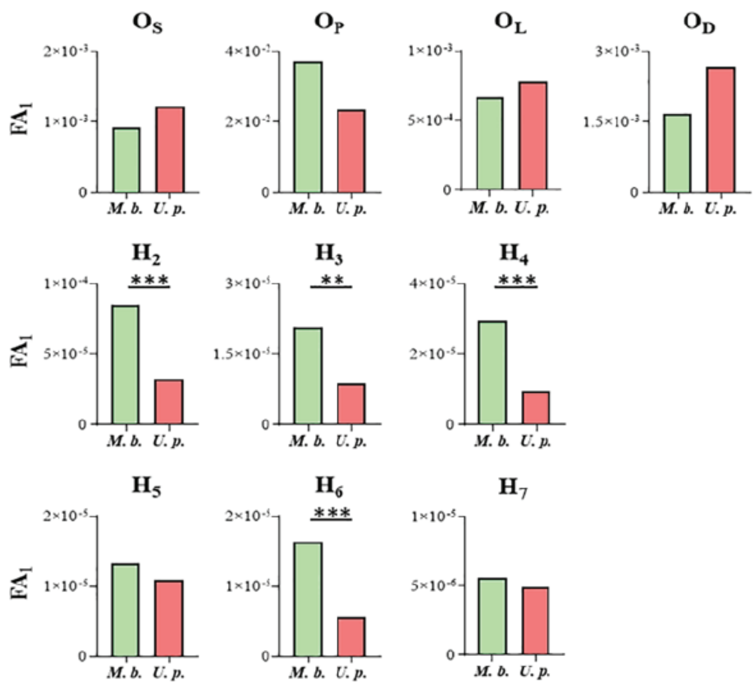

Fig. 2: Comparison of the index FA_1 between the native fish species *Mullus barbatus* (*M.b.*; green) and the alien species *Upeneus pori* (*U.p.*; red). O_L, maximum otolith length; O_D, maximum otolith depth; O_S, otolith surface area; O_P, otolith perimeter; H_2-H_7, harmonics two to seven. Significant differences between the two species are shown with $^{**}p < 0.01$, $^{***}p < 0.001$.

4. Discussion/Conclusion

Existing literature indicates that deviations from perfect bilateral symmetry in otolith morphology may result from different environmental stressors during fish development (expressed as fluctuating asymmetry, FA). For all of the examined otolith traits, the alien species *U. pori* did not exhibit higher bilateral asymmetry in comparison to the native *M. barbatus*. Instead, *M. barbatus* presented significantly higher otolith asymmetry in several shape descriptors. Given that the level of fluctuating asymmetry is a measure of environmental stress (Palmer & Strobeck, 1986), these findings suggest that the examined alien species is comparatively well adapted to its new habitat. In other cases, departures from bilateral asymmetry may arise as a phenotypically plastic response, induced by environmental and/or genetic drivers (expressed as directional asymmetry, DA) (reviewed in Geladakis *et al.* 2021, 2022). The comparatively lower otolith asymmetry of *U. pori* may reflect a strong selection against individuals with high asymmetry and/or other developmental disadvantages (e.g., deformities) within the current (new) environmental conditions, probably during early developmental stages (e.g., larval stages). On the other hand, the significantly higher otolith asymmetry of *M. barbatus* may imply a stressful situation for native species in the Mediterranean Sea under the current trend of increasing sea temperature.

More research is warranted (including the study of alien fish samples at different stages of development and geographical sites), in order to further evaluate the utility of otolith bilateral asymmetry as a tool to assess the adaptation of alien species in their new environments and the chances of establishing abundant populations in the Mediterranean Sea.

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