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Phenotypic Affinities on Fry of Four Mediterranean Grey Mullet Species

George Katselis[1,*], George Hotos[1], George Minos[2], Kosmas Vidalis[1]
[1] Technological Educational Institution (T.E.I.) of Messolonghi, Department of Aquaculture and Fisheries, 30200 Messolonghi, Greece.
[2] Alexandrian Technological Educational Institution (A.T.E.I.) of Thessaloniki, Department of Fisheries Technology and Aquaculture, 63200 N. Moudania, Greece.
* Corresponding Author: Tel.: +30. 263 1058230; Fax: -
E-mail: gkatsel@teimes.gr
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Abstract

Variation in eight morphometric characteristics of the fry of four grey mullets species (*Liza aurata, Liza saliens, Chelon labrosus* and *Mugil cephalus*, Pisces: Mugilidae), all within the total length class of 20-35 mm (the grey mullets fry in this class size are the target of aquaculturists for stocking), in western Greece, was examined by using multivariate analysis. The relatively lower values of coefficients of variance (CV<20%) indicated a high heritability for each character, while the discriminant analysis revealed that about 92.7 % of the examined specimens could be correctly distinguished and classified in the four species. The discriminant analysis and the unweigthed per-group method with arithmetic averaging (*UPGMA*) cluster analysis based on the Mahalanobis distance between group centroids showed that *M. cephalus* and *C. labrosus* were quite similar in morphology, while *L. aurata* and *L. saliens* were rather different. The results of this study revealed that in contrast to the current perception, the analysis of the morphometric variation of mullet fry could be used for their discrimination.

Key Words: fry, grey mullet, multivariate analysis, morphometric.

Introduction

Grey mullets are mainly a catadromous family, excluding a few member species. They live in schools on the coasts, lagoons and rivers of tropical and subtropical waters (McDowal, 1988). They are important food fishes. The euryhalinity, eurythermality and their simpler diet, as well as the rapid growth of some species, have made them the object of aquaculture in many parts of the world, including the Mediterranean (Oren, 1981). In 2003, 42,738 tons of grey mullets (about 60% of their total production) were produced by aquaculture in marine, brackish and inland waters of countries bordering the Mediterranean and the Black Sea (FAO, 2005). Artificial breeding of grey mullet has been practiced since the 1970s (Kuo *et al*., 1973), but most of the fry for the Mediterranean aquaculture are still obtained from wild stocks. Only in Egypt about one billion of wild grey mullet fry (total length about 20-35 mm) have been collected during the last decade to supply aquacultures in marine, brackish and fresh waters (Sadek and Mires, 2000). The mullet fry on total length class size of 20-35 mm are the target of aquaculturists for stocking, because the individuals configure numerous shoals in the coastal water and are easy to capture (Brusle, 1981; Zismann, 1981).

Except for species with a unique attribute, such as an unusually large fin-ray count, it is extremely difficult to distinguish the species of small grey mullet (Thomson, 1997). Also the problem for the identification of the various species in young stages exists due to rapid changes of body proportions (allometry) in their ontogenic stages. A series of meristic features (e.g. number of pyloric caeca, fin elements, pigmentation patterns) have been proposed (Perlmutter *et al.*, 1957; Zismann, 1981; Cambrony, 1984; Serventi *et al.*, 1996; Minos *et al.*, 2002) with a high discretional ability on the identification of grey mullet's young stages. However, the analysis of the morphological variation among the young stages of grey mullet species can provide a significant contribution on their identification. An empirical scale based on the differences of the external morphometric features among the grey mullets fry as a contribution to their identification has been proposed (Cambrony, 1984).

Multivariate analysis of a set of phenotypic characters is regarded as a powerful technique for the determination of morphological relationships between the populations of a species (Claytor and MacCrimmon, 1988; Corti and Crosetti, 1996; Vidalis *et al*., 1997; Mamuris *et al*., 1998; DeVries *et al*., 2002; Palma and Andrade, 2002) and for investigating taxonomic problems between species (Spain *et al.*, 1980; Karakousis *et al.*, 1993; Iliadou *et al.*, 1996; Akyol and Kinacigil, 2002).

The aim of this study is the evaluation of the morphological similarity/dissimilarity of the four grey mullet fry species on total length class of 20-35 mm (the grey mullets fry in this class size are the target of aquaculturists for stocking) in the Western Greece, with the use of the multivariate analysis technique. This evaluation is a contribution to the identification problem of the grey mullet fry.

Materials and methods

Samples were collected using a fine-meshed beach seine in the Messolonghi –Etoliko lagoon in the Western Greece from January 1992 to June 1993 during the period which each species appears (Katselis *et al.*, 1994). The fish of each haul were immediately killed with an overdose of MS–222 and preserved in 4% formalin.

It is known that the body proportions in young fish stages show major changes (allometry) during the growth. Thus, in order to remove the effect of allometry associated with the differences on the size among the species, it is essential to select the same class size for all species (Minos *et al.*, 1995). So, for all species, the selected total length class was of 20-35 mm.

These were used for the analysis of 537 individuals randomly selected from 3-4 monthly samples on total length range of 20-35 mm of five species of the Mugilidae family, namely *Mugil cephalus* (Linnaeus, 1758), *Chelon labrosus* (Risso, 1827), *Liza saliens* (Risso, 1810), *Liza aurata* (Risso, 1810) and *Liza ramada* (Risso, 1810).

Identification of these fish was made according to the characters of the identification keys of Mediterranean grey mullet (Cambrony, 1984; Zismann, 1981; Minos *et al.*, 2002). A series of measurements were recorded on each specimen for 12 distance characters (Figure 1). All measurements were made to the nearest 0.01 mm with electronic vernier; for paired structures only the left structure value was taken.

All the measurements were measured 15-20 days after the collection of specimens.

Due to differences in the scale of the measurements, particularly between the measurements of body lengths (total, fork and standard length) and the measurements on the head, all the data were transformed to natural logarithms (Hair *et al.*, 1998).

The coefficient of variation (*CV*) was computed for each character according to:

$$CV = (100 \times SD) / X_m$$

where *SD* is the standard deviation and X_m is the mean of the transformed measurements of characters in each species. In each species' sample group, morphological variability was estimated by the multivariate generalization of the coefficient of variation (CV_p) according to:

$$CV_p = 100 \times \sqrt{\sum SD_x / \sum M_x} ,$$

where SD_x is the variance of each morphometric variable and M_x is the mean squared (Van Valen, 1978).

A preliminary analysis of covariance (ANCOVA) showed that in all species, the morphometric characteristics in relation to total length presented no significant differences from the isometric.

To identify whether there are any statistically significant differences between the species for each character, a one-way analysis of variance (ANOVA) was performed (Snedecor and Cochran, 1980; Zar, 1984).

In order to elucidate the differentiation of the species, forward stepwise discriminant analysis (*DA*), based on the generalized Mahalanobis distance, was used to determine the similarity between the species and the ability of these characters to identify the specimens correctly.

The percentage of discrimination per pair of species (*PDPS*) was estimated as the proportion of correctly classified individuals of two species on the total classified individuals in two species. In other words, the *PDPS* represented the probability of the correctly identified individuals of two particular species.

An unweighted per-group method with arithmetic averaging (*UPGMA*) cluster analysis (Hair *et al.*, 1998) based on the Mahalanobis distance between the group centroids was applied to determine the similarity/dissimilarity between species.

All statistical analyses were performed using SPSS PC ver. 10.

Results

The analysis of variance (ANOVA) showed significant differences in the mean total length between species ($F = 42.27$, $df = 4.533$, $P<0.05$) while the Tukey test showed that the total length of *L. ramada* was significantly smaller than those of other species. The rest of the species showed no significant differences on the total length (Figure 2). Therefore, *L. ramada* was excluded from the final analysis.

Apart from the standard length (*SL)*, fork length (*FL)* and pre-orbital distance (*PRE)*, the mean values of the remaining characters examined (Table 1) differed significantly (ANOVA, $P<0.05$) amongst the four species of Mugilidae.

Based on the characters, which differed significantly amongst the four species, the *DA* extracted three canonical variables (*CaV*) contributed overall to the variance. The first and second canonical variables contributed 69.8% and 21.7%, respectively, while the third canonical variable contributed 8.5%, to the total variance (Table 2).

The characters of primary importance in distinguishing groups were the maximum body height (*MBH)*, pre-dorsal fin II distance (D_2) and head length (*HL)* for the CaV_1, the postorbital distance (*POSTE*) for the CaV_2 and pre-dorsal fin I distance (D_1), eye diameter *(ED)* and minimum body height (*FH)* for the CaV_3.

The unstandardized coefficients for the eight

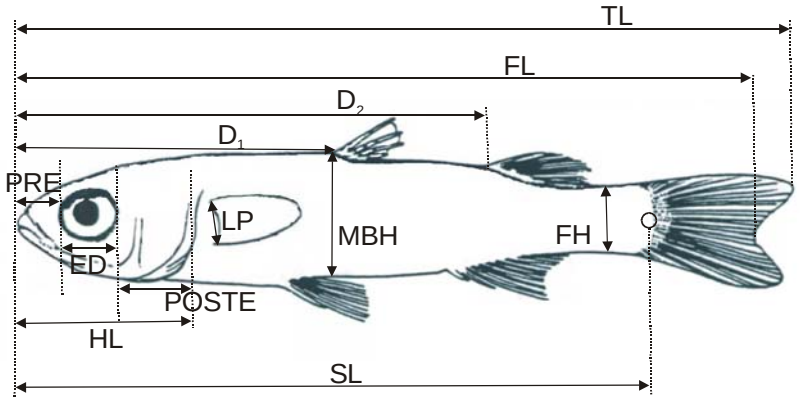

Figure 1. Measurements on the body of grey mullet fry specimens. *TL*: total length; *SL*: standard length; *FL*: fork length; D_1: pre-dorsal fin I distance; D_2: pre-dorsal fin II distance; *PRE*: pre-orbital distance; *ED*: eye diameter; *POSTE*: postorbital distance; *HL*: head length; *LP*: Base of pectoral fin; *MBH*: maximum body height; *FH*: minimum body height.

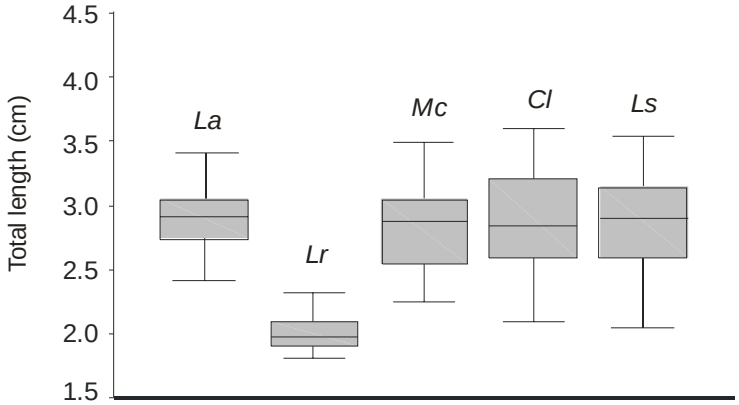

Figure 2. Box and whiskers plot of the total length distributions of five grey mullet fry. *Ls*: *Liza saliens*, *Lr*: *Liza ramada*, *La*: *Liza aurata*, *Cl*: *Chelon labrosus*, *Mc*: *Mugil cephalus.*

Table 1. Descriptive statistics of the raw measurements (in cm), the coefficient of variation (*CV*) of each measurement, the multivariate coefficient of variation of each species (*CVp*) and results of ANOVA based on the transformed data (*Xm* is the mean value; *SD* is standard deviation; NS: no significant differences P>0.05; **: significant differences P<0.05; *Morph. Charact*: abbreviations of morphometric characteristics)

Morph. Charact.	*Liza aurata* N=156 Month: N, D, J & F					*Mugil cephalus* N=102 Month: O, N, D & J					*Chelon labrosus* N=121 Month: M, J & J					*Liza saliens* N=137 Month: A, S, O & N					
	Xm	*SD*	Range		*CV*	*Xm*	*SD*	Range		*CV*	*Xm*	*SD*	Range		*CV*	*Xm*	*SD*	Range		*CV*	
TL	2.90	0.32	2.12	3.80	11.08	2.85	0.45	2.30	4.42	15.67	2.96	0.37	2.10	3.60	12.43	2.89	0.38	1.70	3.75	13.19	NS
FL	2.80	0.31	1.95	3.60	11.12	2.74	0.44	2.20	4.21	15.89	2.83	0.36	2.00	3.42	12.61	2.77	0.37	1.62	3.60	13.27	NS
SL	2.36	0.26	1.76	3.10	10.98	2.29	0.38	1.85	3.63	16.57	2.39	0.30	1.70	2.89	12.53	2.33	0.30	1.39	3.02	13.09	NS
D1	1.15	0.15	0.81	1.57	13.02	1.14	0.22	0.85	1.88	18.85	1.27	0.17	0.85	1.59	13.64	1.19	0.16	0.76	1.64	13.80	**
D2	1.62	0.22	1.12	2.23	13.30	1.59	0.30	1.23	2.61	18.71	1.76	0.24	1.16	2.17	13.79	1.68	0.23	0.93	2.25	13.97	**
LP	0.40	0.04	0.29	0.52	10.50	0.40	0.05	0.32	0.59	13.31	0.43	0.08	0.29	0.56	19.03	0.38	0.06	0.21	0.54	15.09	**
HL	0.61	0.08	0.44	0.83	13.40	0.60	0.12	0.45	1.00	19.35	0.69	0.09	0.46	0.89	13.66	0.64	0.08	0.40	0.84	13.00	**
MBH	0.48	0.06	0.35	0.67	13.15	0.50	0.09	0.40	0.79	17.60	0.54	0.07	0.37	0.73	12.65	0.44	0.06	0.29	0.58	13.96	**
FH	0.24	0.03	0.19	0.33	10.61	0.22	0.03	0.18	0.33	13.65	0.25	0.03	0.19	0.32	12.57	0.23	0.03	0.15	0.29	11.30	**
ED	0.22	0.02	0.18	0.26	6.86	0.21	0.03	0.18	0.30	12.61	0.23	0.03	0.17	0.29	13.22	0.21	0.02	0.14	0.27	10.80	**
PRE	0.14	0.02	0.10	0.20	13.96	0.14	0.03	0.10	0.22	19.88	0.14	0.02	0.10	0.18	13.24	0.14	0.02	0.09	0.20	14.27	NS
POSTE	0.24	0.04	0.16	0.35	17.15	0.25	0.07	0.16	0.50	27.16	0.32	0.06	0.19	0.46	19.33	0.28	0.05	0.14	0.39	18.02	**
CVp					11.47					16.51					12.84					13.33	

variables of the morphometric characters for each of the discriminant function (canonical variable) are shown in Table 2. These discriminant functions identified the membership (classification) of individual fish in the data with one of the four species (Table 3) with a success rate of 92.7% (Table 4). The graphical presentation of the first and second canonical variables is shown in Figure 3. The percentage of discrimination per pair of species (*PDPS*), except some cases, was very high (>95%). The highest value of *PDPS* was shown between *L. aurata* and *C. labrosus* (100 %), while the smaller value of *PDPS* was shown between *M. cephalus* and *C. labrosus* (88.9%). The *PDPS* values of the rest of the combinations fluctuated between 95-99% (Table 5).

The *UPGMA* cluster analysis based on the Mahalanobis distance between group centroids showed that the four species were clustering in two clusters. The *L. aurata*, *C. labrosus* and *M. cephalus* belong to the first cluster (cluster I) while the *L. saliens* belongs to the second one (cluster II) (Figure 4). Moreover, the *C. labrosus* and *M. cephalus* show greater morphological similarities than *L. aurata* and *M. cephalus.*

Discussion

It is certain that the parameters related to the allometric growth of fishes and the timing of the sampling, could impose some major limitations for the study of morphological relationships among the species.

The use of the morphometric characters to distinguish young stages of the grey mullet species is a method with low accuracy due to major changes of the body proportions (allometry), which occur in these stages (Thomson, 1981). In this case, the use of

Table 2. Results of Discriminant Analysis (*DA*) based on the transformed data, and unstandardized coefficients of each morphometric variable on three canonical variables (CaV_i)

	CaV_1	CaV_2	CaV_3
% of variance	69.8	21.7	8.5
Characters	Discriminant Function Coefficients		
D1	2.2	-0.2	-13.3
D2	-12.4	-9.4	-4.2
LP	5.2	1.8	-4.2
HL	-11.9	-4.2	3.5
MBH	19.5	4.3	-1.3
FH	-1.5	-8.1	12.3
ED	-1.8	-8.1	10.6
POSTE	1.1	15.9	4.2
Constant	16.5	4.5	40.7

Table 3. Mean canonical variables (CaV_i) of each species of four grey mullet fry (centroids) based on the transformed data (the standard deviation in parenthesis)

Species group	CaV_1	CaV_2	CaV_3
Liza aurata	0.74 (1.67)	-2.09 (1.55)	0.33 (1.7)
Mugil cephalus	1.98 (1.81)	0.34 (1.6)	-0.84 (1.9)
Chelon labrosus	1.41 (1.7)	1.04 (1.88)	1.02 (2.1)
Liza saliens	-2.06 (2.3)	0.20 (2.3)	-0.11 (2.1)

Table 4. Results of discriminant analysis classification showing the percentage of specimens classified in each group

Species	Groups				Total number of specimens
	1	2	3	4	
Liza aurata	93.2	6.8	0.0	0.0	156
Mugil cephalus	0.0	89.9	10.1	0.0	102
Chelon labrosus	0.0	11.9	86.2	1.9	121
Liza saliens	1.7	1.7	0.8	95.8	137

Total number of specimens correctly classified: 92.7%

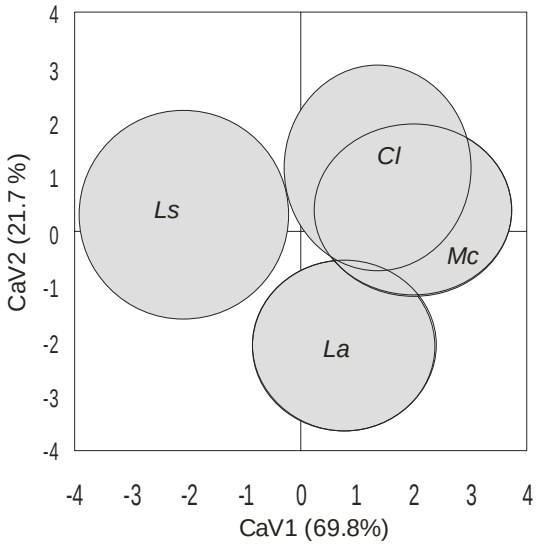

Figure 3. Discriminant analysis plot where the 8 morphometric variables were used. *Ls*: *Liza saliens*, *La*: *Liza aurata, Cl*: *Chelon labrosus, Mc*: *Mugil cephalus*. Ellipses include 95 % of the specimens.

Table 5. Percentage of distinguishing per pair of species (*PDPS*) based on results of discriminant analysis classification

	Liza aurata	*Mugil cephalus*	*Chelon labrosus*	*Liza saliens*
Liza aurata	0			
Mugil cephalus	96.42	0		
Chelon labrosus	100.00	88.89	0	
Liza saliens	99.11	99.09	98.54	0

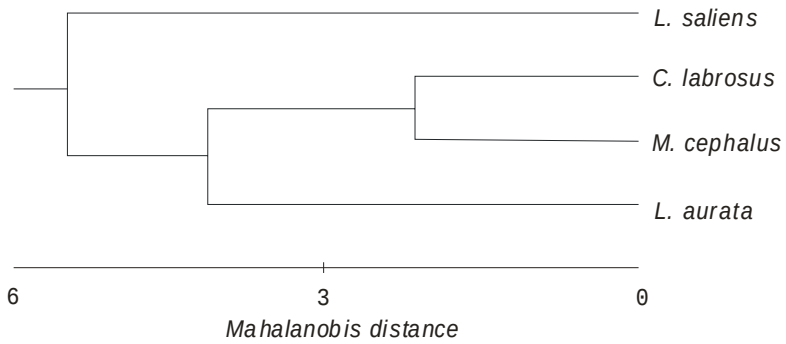

Figure 4. *UPGMA* cluster analysis based on the Mahalanobis distance between the species centroids.

the same body size of individuals of the compared groups (species) overcomes the problem, but the findings on this size are limited. In the present study, this problem has been overcome with the use of the total length class of 20-35 mm for all species.

The fact that each species individuals were collected at various times of the year could create morphological groups due to different dimensions related to gonad development and stomach fullness of fishes. Certainly, the gonad developments of the four mullet species begin after the second year of their lives while the sizes of members of each species on this study correspond to a few months of their life (Oren, 1981). Because the object of this study is associated with the various periods in which fry of each species appear in the coastal ecosystems (Katselis *et al*., 1994), the collection of each species at various times of the year is ineluctable. However, the fact that the final sample of each species was provided from the 3-4 monthly samples (Table 1) minimizes the likelihood of appearance of one morphological group associated with stomach fullness of the fishes, but with the possible increasing of the characters variation.

The morphometric characters (phenetic characters) are the composite effect of genotype and environmental factors and are under the influence of natural selection (Dobzansky, 1970). Table 1 shows that the within-species variation was less evident as indicated by the relative low *CV* values (*CV*<20%) for each character and it suggests that each species consists of a phenotypicaly homogeneous group. Considering that there should be a negative correlation between *CV* and estimated heritability of morphological characters (Soule and Couzin-Roudy, 1982), the relatively low value of *CV* found in the present study indicates a high heritability for each character.

According to *DA* classification, 92.7% of the specimens examined in this study, can correctly be classified into four species. The characters of primary importance in distinguishing groups were the maximum body height (*MBH*), pre-dorsal fin II distance (*D2*) and head length (*HL*) for the CaV_1, the postorbital distance (*POSTE*) for the CaV_2 and pre-dorsal fin I distance (*D1*), eye diameter (*ED*) and minimum body height (*FH*) for the CaV_3.

The position of each species on the first two canonical variables (CaV_1 & CaV_2) supported a rank based on profile of each species (Figure 3). Considering that all the examined species had equal (statistical) length, the ranking of species on the CaV_1 supported a slender shape of *L. saliens* (left) and more compact shape of *M. cephalus* (right) resulting from the lower body height (0.44±0.06 cm) and higher head length (0.64±0.08 cm) of *L. saliens* than those of *M. cephalus* (Table 1: *MBH*= 0.50±0.09 cm; *HL*= 0.60± 0.08 cm). These findings are in agreement with the profiles descriptions of grey mullet fry according to Cambrony (1984).

The present study confirms the morphological differences between the grey mullet fry and that the species are clearly discriminated on the basis of their external features. The identification of these species using the external morphometric features can be achieved, in practice, using the unstandardized coefficients for eight variables of the morphometric characters (natural logarithm of raw data) for each of discriminant function, which is showed in Table 2. Calculating the CaV_1, CaV_2 and the CaV_3 and using the values of the Table 3, it is easy to find out (with likelihood about of 92.7 %) to which species some particular external morphometric features belong. However, as shown in Table 5, apart from the *M. cephalus* and *C. labrosus*, all the other species using the set of morphometric features of Table 2, can be separated easily. According to some authors (Bograd, 1961; Brusle 1981; Cambrony, 1984; Katselis *et al.*, 1994; Hotos, 2003), the fry of *C. labrosus* at a range length of 20-35 mm appears on the Mediterranean coastal lines during the periods of transition from spring to summer (May to July) while the fry of *M. cephalus* appears during the seasons of autumn and winter (September to December), respectively. So, it is clear that the problem of the proposed method to identify successfully the individuals of the *M. cephalus* and *C. labrosus*, has been overcome due to different periods of availability of these species.

The cluster analysis based on the Mahalanobis distance (Figure 4) has provided an integrated measure of species interrelationships and has shown that affinities with genera are not reflected in their body forms. The classification of the grey mullet fry species based on external morphological features differed from this in the adult specimens. According to Akyol and Kinacigil (2000), discriminant analysis in seven morphometric characters (included in this study) in adult specimens of grey mullets showed that *L. saliens* and *L. aurata* were similar in form, while *M. cephalus* and *C. labrosus* were rather different. Also, information on the morphological measurements on the *L. saliens* and *L. ramada* (Minos *et al.*, 1994; 1995) supports this classification pattern. This fact can be explained with the differences of body proportions between the young and adult stages of fishes. However, while there is support to the findings on the adult specimens of 11 species of Australian grey mullets (Spain *et al.*, 1980), the high relation between the genera and body form on the Mugilidae species is not expected.

In conclusion, the results of this study revealed that, in contrast to current perception, the analysis of the morphometric variation of mullet fry with total length range 20-35 mm, can be used for their clear discrimination. However, due to participation of a major number of morphometric features on the clear discrimination of grey mullets fry, their identification in the field, based on these features, is rather difficult. On field routine, the general impression is that the results of this study (e.g. slender or massive body), when accompanied by information regarding the season, in which fry of the species appears, as well as the meristic features (e.g. number of pyloric caeca, fin elements, pigmentation patterns), can be a significant contribution for species identification.

On the other hand, the evaluation of morphological variability of grey mullet fry provides the ability to develop modern tools for their identification. For example, the results of this study (particularly the ones in Table 3 and Table 4), when accompanied by information regarding the season, in which fry of the species appears, can be used as a basis for the development of a software routine for the identification of grey mullets fry.

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