

1 Phenotypic variability in populations of red mullet (*Mullus barbatus* L.,
2 1758) across different environments in the Central Mediterranean across
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25 Abstract

26

27 Geometric morphometrics is an effective tool for investigating intraspecific variations of fish body
28 shape and contributing to discriminate fishery stocks. This study applied geometric morphometrics to
29 examine the patterns of intraspecific morphological differentiation among four red mullet (*Mullus*
30 *barbatus* L., 1758) local populations in the Strait of Sicily (Central Mediterranean). It was hypothesized
31 that morphological differences among local populations caused by the diverse hydrodynamic features
32 characterizing the sampled areas and maintained by physical isolation determined by high geographical
33 separation. It was also assessed whether, body shape homogeneity/variability within local populations
34 was the result of the different seabed rugosity (used as a proxy of habitat complexity). Results revealed
35 that morphological divergence between local populations was constrained by short the geographical
36 separation. The differences observed mainly concerned the fish body depth, with more elongated
37 specimens inhabiting high hydrodynamic pattern areas. Moreover, homogeneity in body shapes within
38 localities was positively related with the seabed rugosity, reflecting a reduction of intraspecific
39 morphological variability in habitats of higher structural complexity. The improved understanding of the
40 spatial structure of red mullet populations in the Strait of Sicily may be valuable for aligning the
41 definition of fishery stock units to the boundaries of natural populations.

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43 Keywords: geometric morphometrics; phenotypic differentiation; Kinetic energy; habitat complexity;
44 Strait of Sicily; morphospace patterning

45

46 **Significance Statement**

47 Morphological differentiation among four red mullet local populations in the South-Central
48 Mediterranean was examined using geometric morphometrics. Morphological differences were
49 hypothesized to be caused by diverse hydrodynamic features and maintained by geographical distance.
50 Whereas, morphological homogeneity within local populations, was hypothesized to be a result of the
51 habitat complexity constraining the morphological variability. Overall, high structural complexity
52 promotes low variation in body morphology and geographical isolation constrains the diversification
53 originating from different hydrodynamic conditions.

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56 Introduction

57

58 Understanding the relationships between the phenotype of an organism and its environment
59 represents one of the main questions in evolutionary biology and ecology, and it has been the aim of
60 numerous studies since early work on the 'phenotypic variation' by Darwin (1859). Nowadays, it is
61 recognized that morphological traits of organisms can be modulated by several biotic and abiotic
62 factors (e.g. Schluter, 2000; Meyers & Bull, 2002; Fulton *et al.*, 2013). In aquatic environments, these
63 include temperature, salinity, dissolved oxygen, water current velocity, habitat structural complexity or
64 predation intensity. These factors can exhibit great variability across space and time and have the
65 potential to induce morphological diversification in fish species either directly, as a response to distinct
66 environmental conditions, or via behavioral plasticity i.e. through the acquisition of adaptive behaviors
67 aimed at optimizing essential biological and ecological functions such as feeding, predator avoidance,
68 or reproduction (Svanback & Eklov, 2002; Olsson *et al.*, 2007; Langerhans & Reznick, 2010).
69 Morphological diversification along environmental gradients has been observed at both interspecific
70 (Neat & Campbell, 2013; Sampaio *et al.*, 2013; Montaña *et al.*, 2014; Farré *et al.*, 2015) and intraspecific
71 (Langerhans *et al.*, 2003, 2007; O'Reilly & Horn, 2004; Garduno-Paz *et al.*, 2010; Fruciano *et al.*, 2011;
72 Sampaio *et al.*, 2013; Bracciali *et al.*, 2016) level within fish assemblages.

73 For instance, habitat complexity is considered an environmental component triggering, both directly
74 and indirectly, the acquisition of peculiar morphological characters of species (Garduno-Paz *et al.*, 2010;
75 Langerhans & Reznick, 2010; Farré *et al.*, 2015). Direct effects may be due to the physical characteristics
76 of the habitat; e.g. in laboratory experiments, a more elongated body and a small head size were
77 observed in specimens of threespine sticklebacks exploiting preys in the interstitial spaces of complex
78 rocky habitat, compared to specimens exposed to gravel habitat (Garduno-Paz *et al.*, 2010). Indirect
79 effects may be linked to the influence of habitat complexity on biotic interactions (Montaña *et al.*, 2014;
80 Farré *et al.*, 2015) and consequently on individuals' behaviours, such as feeding strategies (Svanbäck &
81 Eklöv, 2002; Costa & Cataudella, 2007). In fact, more complex structural habitats are generally
82 associated to greater biodiversity (García-Charton & Pérez-Ruzafa, 2001; Thrush *et al.*, 2001; Danovaro
83 *et al.*, 2010; Kovalenko *et al.*, 2012; Farré *et al.*, 2015) which implies more intense competition for the
84 resources use. Therefore, diversification in feeding behaviour and body morphology could favour
85 trophic niche partitioning between and within species (Swanson *et al.*, 2003; Sampaio *et al.*, 2013),
86 which ultimately plays a key role for specific sustainable competition and coexistence within

87 communities (Swanson et al., 2003; Sampaio et al., 2013; Montaña et al., 2014). Consistent with this
88 expectation, experimental evidence has confirmed that the increase of intraspecific competition within
89 a natural population of threespine sticklebacks increased both the degree of niche variation and the
90 strength of diet-morphology correlation (Svanback and Bolnick, 2007). In addition, there is evidence
91 that morphological changes following niche shifts occur on a short time-scale (Wanink & Witte, 2000).
92 Much attention in the literature has been also paid to the adaptive significance of the effects of currents
93 flow velocity on the differentiation of fish body plan. Indeed, fish morphology is directly related to
94 swimming capabilities, differentially selected in response to varying biotic and abiotic factors
95 (Langerhans & Reznick, 2010; Bruckerhoff & Magoulick, 2017). The literature suggests that a more
96 streamlined body shape, fusiform body with narrow caudal peduncle region, is favored in high-currents
97 habitat. This allows fish to enhance steady state (constant-speed locomotion) swimming capabilities
98 aimed to reduce drag and minimize the energetic costs of swimming while performing routine tasks
99 (Domenici, 2003; Langerhans & Reznick, 2010; Bruckerhoff & Magoulick, 2017). Conversely, unsteady
100 (with rapid bursts and turns) swimming capabilities, enhanced by a deep body with large caudal fin,
101 allow fish to exploit strategies requiring high acceleration or maneuverability and are mostly developed
102 in low-current habitats (Langerhans & Reznick, 2010; Bruckerhoff & Magoulick, 2017). In agreement
103 with this general pattern, intraspecific differences in body shape across gradients of water velocity have
104 been documented, on a small spatial scale of few kilometers, in Mediterranean damselfish (*Chromis*
105 *chromis*) (Bracciali et al., 2016). The authors observed the development of body traits useful for
106 propellant swimming, including a more fusiform body shape in a high-currents habitat, where fish need
107 to remain stable in the water column to detect prey. By contrast, a greater body height and longer
108 pectoral fins, which assure greater maneuverability for the active search of prey, were recorded in
109 individuals inhabiting low-currents habitat (Bracciali et al., 2016).

110 Patterns of morphological variations across environmental gradients can indicate groups of fish that
111 are isolated enough to maintain phenotypic differences (e.g. Medina *et al.*, 2008; Cadrin *et al.*, 2010),
112 being the persistence of these differences a signal of limited mixing and adaptive phenomena which
113 may have also a genetic basis (Cadrin, 2010; Cadrin *et al.*, 2010). Identifying the population units (or
114 stock units) which exhibit morphological homogeneity across a geographical region is therefore
115 essential for fishery resource management. Indeed, ideally, any fishery management plan (for both
116 exploited or threatened species) should target to fish stock units with such a level of demographic

117 independence to be considered self-sustaining on an ecological time-scale (e.g. Eagle *et al.*, 2008;
118 Cadrin, 2010). Actually, it is widely recognized that misalignments exist between the spatial structure
119 of biological populations and the definition of stock units used in assessment and management of
120 harvested species, mainly deriving from inadequate knowledge of populations spatial structure. To fill
121 this gap, in the last decades, several approaches have been used for identification of stock units and
122 geographical boundaries of exploited fish species (Cadrin *et al.*, 2014), including analyses of
123 morphometric characters. Specifically, landmark-based geometric morphometric (Rohlf & Marcus,
124 1993; Bookstein, 1997) has emerged as powerful method to analyze morphological differences
125 between populations and has been applied for providing information on fish population structure in
126 relation to geographic range, environmental variability or connectivity (Murta, 2000; Cadrin, 2000;
127 Silva, 2003; Langerhans *et al.*, 2003; Turan, 2004; Turan *et al.*, 2006; Medina *et al.*, 2008; Cadrin *et al.*,
128 2010; Mahe *et al.*, 2014). The technique can be also usefully applied to detect intra-population
129 morphological variability, i.e. among individuals within single local populations in a specific
130 environment. Indeed, habitats suffering a high degree of disturbance (e.g. low complex soft sediment
131 habitats; Norkko *et al.*, 2010) and hence characterized by unpredictable and variable conditions, are
132 expected to promote higher variation in the morphological characters of fish (e.g. Bruckerhoff &
133 Magoulick, 2017) because high variability in environmental conditions experienced during the life of
134 fish prevents the accumulation of specific adaptations within a local population (e.g. Schluter, 2001;
135 Bruckerhoff & Magoulick, 2017). By contrast, higher environmental stability, as well as increasing
136 habitat complexity, could constrain the morphological variability favoring the development of common
137 local morphological features (Montaña *et al.*, 2014) especially those related with feeding (e.g. Svanbäck
138 & Eklöv, 2002; Costa & Cataudella, 2007) or locomotion (e.g. Blake, 2004) strategies both for groups of
139 species within assemblages (e.g. Farré *et al.*, 2015) or within a single species (e.g. Garduno-Paz *et al.*,
140 2010).

141 In this study, we focus on populations of red mullet (*Mullus barbatus* L., 1758) of the Strait of Sicily
142 (south-central Mediterranean). Red mullet is a benthic fish with marked preferences for gravel, sandy
143 and muddy bottoms (Voliani *et al.*, 1999) along the continental shelf (from 5 to 250 m of depth),
144 although a wider bathymetric range has been reported in some Mediterranean areas. It is one of the
145 most valuable commercial species in the Mediterranean Sea where it is frequently fished by trawlers
146 and small-scale fisheries using a variety of gears (Tserpes *et al.*, 2002). Along the northern part of the

147 Strait of Sicily (SoS), the red mullet annual landings reach on average 774 tons (from 2006 to 2015,
148 GFCM report 2016). Inside the SoS, the occurrence of different populations between the Sicilian
149 (Northern Strait) and the African shelves has been argued during the past decades (Levi *et al.*, 1992,
150 1995). Furthermore, the identification of persistent and discrete spawning and nursery areas (Garofalo
151 *et al.*, 2004, 2008) coupled with the complex topography and water circulation (Béranger *et al.*, 2004)
152 led to consider the existence of different units of red mullet highly probable (Fiorentino *et al.*, 2008).
153 Recently, these hypotheses were strengthened by a study aimed to assess the connectivity patterns
154 between spawning and nursery areas across the SoS (Gargano *et al.*, 2017). The complex sea circulation
155 of the SoS modulates the dispersal of eggs and larvae among habitats and represents an important
156 factor for a species that presents a larval stage restricted to surface waters such as red mullet. However,
157 the results of the study showed a weak connectivity between northern and southern side of SoS, and
158 most of the local units seemed to be self-recruiting populations (Gargano *et al.*, 2017), likely favouring
159 a pattern of morphological diversification of red mullet populations in response to local environmental
160 factors. This makes the red mullet of the Strait of Sicily an ideal case study for applying a landmark-
161 based geometric morphometric approach to assess and compare body morphology between and within
162 different localities in the area. Specifically, we i) tested the difference in body shapes among local
163 populations from four localities with different hydrodynamic conditions; ii) assessed if geographical
164 separation between local populations constrains morphological differentiation; iii) investigated the
165 homogeneity of body shapes within each locality in relation to sea bottom rugosity used as proxy of
166 habitat complexity.

167 Despite morphological differences between localities would not necessarily indicate the existence of
168 different red mullet populations in terms of self-recruiting populations, this study is expected to provide
169 some insight into the spatial scale of the population structure and the probable phenotypic units of the
170 adult fraction and could contribute to the development of effective management recommendations for
171 this species.

172 Material and Methods

173 **Sampling**

174
175 A total of 153 specimens of red mullet were collected in three localities of the Strait of Sicily (SoS) and
176 in an adjacent area (Fig. 1) between September and October 2013: 43 from Levanzo Island (Le.I.) located

177 off the north-western corner of Sicily, 50 from South-Western-Sicily (S.W.S), 21 from South-Eastern
178 Sicily (S.E.S) and 39 from Lampedusa Island (La.I.) located on the African platform. Samples from Le.I.
179 and La.I., both at *ca.* 95 m of depth, were acquired from professional fishers operating bottom trawling,
180 while samples from S.W.S. and S.E.S., both at *ca.* 40 m of depth, were sampled during the experimental
181 bottom trawl survey MEDITS (Mediterranean International Bottom Trawl-Surveys; Bertrand *et al.*,
182 2002) carried out in the SoS. The northern part of the Strait of Sicily is characterized by two wide shallow
183 banks in the eastern and western parts of the Sicilian coast connected by a narrow continental shelf in
184 the central part. The shelf is irregular and characterized by the terrigenous material carried by the
185 Atlantic Ionian Stream (AIS) and which accumulates mainly near the coast of Sicily (Colantoni *et al.*
186 1985). Different semi-permanent oceanographic features (e.g. eddies, vortices and upwelling areas)
187 driven by the variability of the AIS (Robinson *et al.* 1999, DI Lorenzo *et al.* 2017) are known to occur in
188 the study area, which is also characterized by a high heterogeneity in biocenosis inhabiting their
189 bottoms. Specifically, as regards our sampled areas, the coastal terrigenous muds characterize the
190 south western and the south eastern Sicily, while the other two sampled locations were characterized
191 mainly by compacted muds (assemblage type characterization from Gristina and Interbartolo, 2013).

192 **Morphological data acquisition and analysis**

193
194 For all specimens captured, an image of the left body side was photographed together with a scale bar
195 in order to scale in subsequent analysis each specimen without worrying about its distance from the
196 lens.

197 Techniques of Geometric Morphometrics (GM) were applied to process the digital images and obtain
198 the morphometric characteristics of each specimen. Specifically, we positioned both anatomical
199 landmarks, which capture the body form of the structure under study, and semilandmarks, which are
200 points representing homologous curves and surfaces that define the boundary of the structure under
201 study (Bookstein, 1997; Zelditch *et al.*, 2012). Semilandmarks can be treated as and combined with
202 landmark points using sliding procedures (Zelditch *et al.*, 2012) because they can be statistically treated
203 as normal landmarks along all the morphometric processes. The semilandmarks are in fact obtained by
204 sliding these points along a curve (i.e. the anterior part of the head in this study) so that their spacing
205 does not impact the differences between forms (Webster & Sheets, 2010)

206 The configuration of the body shape of each individual was captured as follow:

207 - 11 homologous landmarks positioned on the left side of the body of each specimen in order to
208 capture the major body characteristics (Fig. 2)

209 - 4 sliding semilandmarks positioned to capture features relevant to the anterior part of the head
210 (Fig. 2).

211 The choice of the position of these landmarks and semilandmarks was taken basically because they best
212 represent the external shape of individuals and have been widely used in other studies (e.g. Costa and
213 Cautadella 2007; Fruciano et al. 2011; O'Really and Horn, 2004). Overall two categories of landmarks
214 and semilandmarks were identified: the first category identifies the position of contact of two different
215 tissues (landmark from 3 to 10); the second instead, identifies categorical points within specific
216 structures of individuals (landmark 1, 2 and 11; semilandmarks from 12 to 15) (e.g. Slice et al. 1996).
217 Both landmarks and semilandmarks were digitized using TpsDig version 1.40 (Rohlf, 2005).

218 By using Generalized Procrustes superimposition (GPA – Rohlf and Slice, 1990), the *x-y* coordinates
219 obtained, were rotated, scaled and superimposed to a common coordinate system generating the
220 Procrustes shape coordinates containing only information about shape (GPA-Rohlf and Slice, 1990).
221 GPA was performed using tpsRelw version 1.45 (Rohlf, 2007). Although specimens were carefully placed
222 under the camera according to a standardized procedure in order to avoid shape distortions along the
223 morphometric analytical process, they can suffer from some dorsoventral bending which could
224 determine differences not representing true shape changes (i.e. arching effect, Valentin *et al.*, 2008).
225 We removed this arching effect by projecting the shape descriptors onto a vector (Burnaby's orthogonal
226 projection) that modeled the shape changes associated with bending (Valentin *et al.*, 2008).

227 Changes in the arching-free fish shapes were summarized and visualized using relative warp analysis
228 (RWA; Rohlf & Marcus, 1993). RWA is analogue to principal components analysis. This procedure
229 produces different morphological axis (Principal components or Relative warp axis) in a way that best
230 explain the variance in body shape among individuals. Specifically, the first warp is aligned with the
231 direction of the maximal variance of the shapes and the second which is orthogonal to the first will be
232 aligned with the next greatest variance, and so on. The result of this procedure is a set of morphological
233 axes (relative warps) representing morphological characteristics (e.g. Webster & Sheets, 2010).

234 The most informative relative warps that explained the bulk of the overall morphological variation were
235 retained based on both the visual inspection of the variance explained by the relative warps and the
236 *Kaiser-Guttman criterion*. This criterion is based on the eigenvalues, which tell us how much a principal

237 component is able to explain our initial data set (e.g. Magnan *et al.*, 2014). These most informative
238 warps retained were used to test the differences in shapes among localities.

239 **Body shape differences between localities with varying currents velocity regimes**

240 To investigate the effects of water flow velocity on the fish morphologies, we described the
241 hydrodynamic patterns of the different localities by using the average of the sea kinetic energy (KE)
242 during the seven years (2008-2013 up to August) preceding the sampling date at 40 m (S.W.S and S.E.S.)
243 and 90 m (Le.I. and La.I.) depth. We tested pairwise differences of the most informative warps
244 explaining the 55.5% of the shape variation in the overall dataset [RW1 (27.2 %), RW2(15.5%),
245 RW3(12.8%)] among hydrodynamic regimes by using the multivariate linear model with residual
246 resampling (Collyer *et al.*, 2015; Warton *et al.*, 2016) and performing n= 999 iterations. . We used KE
247 with the only purpose to describe the flow regime of the sampled areas. KE gives information on the
248 hydrodynamic regime (Ruiz *et al.*, 2013) and is calculated as the half of the sum of the squares of the
249 horizontal (Zonal) and vertical (Meridional) currents components, both obtained from the marine data
250 portal of the Copernicus Marine Environment Monitoring Service (CMEMS)
251 (<http://marine.copernicus.eu/>).

252 These analyses were achieved by using the 'mvabund' package (Wang *et al.*, 2014).

253 **Relationship between morphological differences and geographic separation**

254 The Euclidean distance between the average scores of the first relative warp (RW1) of localities taken
255 in pairs was used as an indicator of morphology divergence and related, by using simple linear
256 regression, to the spatial separation index (S_{db}) of the sampled locations (*sensu* Medina et al. 2007).
257 The S_{db} was obtained by integrating the distance (d) in nautical miles (nm) and the average bathymetry
258 (b) in meters (m) between sampled locations as follows:

$$259 \quad S_{db} = d \times b$$

260 Since the geographical separation between localities could regulate divergence (Medina *et al.*, 2008;
261 Cadrin *et al.*, 2010), we should observe at least an increasing divergence pattern with the rise of the
262 physical separation. The uncertainty (i.e. the confidence intervals) of the regression parameters was
263 estimated by bootstrap of the model residuals. Specifically, the residuals of the fitted model were
264 randomized to the fitted values to reconstruct a new response variable which was then regressed to
265 the original explanatory variable (i.e. geographical distance). This procedure was repeated n=999 times

266 and successively the 95% confidence intervals of bootstrapped estimates of the intercept and slope
267 were calculated.

268 **Morphospace patterning and relationship with of habitat complexity**

269 Morphospace was constructed by plotting the first and second relative warps which provided higher
270 morphological variance (e.g. Tuset *et al.*, 2014; Farré *et al.*, 2016). Inside the morphospace the level of
271 morphological variability within localities was assessed by describing the pattern of distribution of the
272 shapes of the specimens coming from each locality i.e. whether it was clustered, dispersed or random
273 (patterning; Shen *et al.*, 2008; Ricklefs, 2012; Tuset *et al.*, 2014). Point pattern analysis was carried out
274 by constructing a grid-based model (or quadrant analysis) over the whole convex-hull (area enclosing
275 all the individuals from all localities) and by counting the number of specimens coming from each
276 locality per quadrant (e.g. Tuset *et al.*, 2014). The choice of the quadrant size can affect this kind of
277 analysis. Large quadrants, in fact, can produce a coarse description of the pattern, while too small
278 quadrants may contain only one point, or they might not contain any points at all (Kalkhan, 2011).
279 Because this reason we used, as a general rule of thumb, the area of a square equal to twice the
280 expected frequency of points in a random distribution (i.e. $2 * \frac{Area}{n}$) where n is the sample size (Kalkhan,
281 2011). After proceeding, the more appropriate grid cells dimension in our analysis were equals to 1/12
282 of the range of the first relative warp. However, to check if the pattern was masked by the number of
283 quadrants, we also performed the same following analysis with the size of the grid cells equal to 1/16
284 and 1/10 of the range of the first relative warp, obtaining very similar results (not presented here). The
285 spatial pattern of the shapes in the morphospace was evaluated by using the Standardized Morisita
286 Index (SI_m ; Smith-Gill, 1975), which is the scaled Morisita Index (Morisita, 1959), assumed to be a very
287 good measure of spatial dispersion because the sample size does not affect it (Krebs, 1999). It allows
288 determining whether a distribution of individuals is grouped together more or less closely than a
289 spatially random distribution. Clumpedeness index (mclu) and the uniform index (muni) were
290 calculated in order to obtain the SI_m by rescaling Morisita index (Smith-Gill, 1975). The SI_m varies from
291 -1 to 1. In uniform patterns, SI_m is < 0 , while in the clustered pattern it has values bigger than 0. Instead,
292 for a random pattern, SI_m value equals to 0. To test if the dispersion pattern of shapes within the
293 morphospace differed significantly from the random expectation, the critical values of the Chi-squared
294 distribution with $n-1$ degrees of freedom were used (e.g. Krebs, 1999). . Low shape variability (clumped

295 pattern inside the morphospace) was considered to be the result of the influence of the high structural
296 complexity of the habitat favouring or constrain specific traits. Hence, we used linear regression and
297 the bootstrapped residuals to test for a positive relationship between the distribution patterns (*i.e.* the
298 S/m) and the habitat structural complexity described by the rugosity index of the seabed. This latter is a
299 quantitative measure of topographical heterogeneity and roughness of the seafloor (Dunn & Halpin,
300 2009), as such it is largely used as a proxy of substrata and surrogate of benthic biodiversity when data
301 on habitat types are not available (Pittman *et al.*, 2007; Dunn & Halpin, 2009). Rugosity ranges from 0
302 (no topographical heterogeneity) to 1 (complete seafloor topographical heterogeneity and roughness
303 variation) and it was calculated from the continuous digital map of depth provided by the MARSPEC
304 database (Sbrocco & Barber, 2013), using the Benthic Terrain Modeller tool (Walbridge *et al.*, 2018) in
305 ArcGIS 10.

306 It is important to keep in mind that for both the univariate analysis performed (*i.e.* Linear regression
307 models) the sample size was small, and consequently these relationships should be considered only for
308 the purpose of exploring the general morphological pattern in relation to the distance between
309 localities and the rugosity, respectively.

310 All the statistical analyses were performed by using the software R (R Core Team, 2016).

311 Results

312 The first three components from the relative warp analysis (RWA) on landmark data were retained and
313 together explained 55.5% of the shape variation in the overall dataset. RW1 (27.2 % of total variance)
314 represented a gradient from deep-bodied to elongate shapes (Fig. 3). The contrast was evident within
315 the head region characterized by a decrease of head depth from low to high values of the RW1 (Fig. 3).
316 Furthermore, the elongation in the caudal region, even if less evident, can be appreciated (Fig. 3) when
317 moving from the minimum to the maximum extreme of the RW1.

318 The RW2 (15.5 % of total variance) differences, principally at the posterior region, in the origin of the
319 second dorsal fin which was more caudally positioned with respect to the overall body in
320 correspondence of the maximum value of RW2 (Fig. 3). The RW3 (12.8 % of total variance) was related
321 to the position of the mouth which was more ventrally positioned at extreme positive RW3 values (Fig.
322 3). Visual inspection of the RW1-RW2 (42.7 % of variance) shape space (Fig. 4) revealed that specimens
323 from S.E.S. were mostly situated at negative values of the RW1; oppositely specimens coming from the

324 La.I. occupied the positive values of the RW1. Whereas specimens from Le.I. and S.W.S. were positioned
325 at intermediate values of RW1 (Fig. 4). Shapes distribution along the RW2 did not show, instead, a clear
326 separation pattern (Fig. 4).

327 **Body shape differences between localities with varying currents velocity regimes**

328 Multivariate analysis of variance between localities showed significant differences in all pairwise
329 comparisons excepting between Le.I. and S.W.S in the three warps analysis (Fig. 4, 5; Table I). Specimens
330 of the S.E.S (lowest values of KE; Fig. 6), had a more rounded and higher head profile than specimens
331 from other areas (Fig. 4, 5; Table I) and overall the change was expressed by an anterior and upward
332 inflation of the head (landmarks 1 and 2, semilandmarks from 12 to 15) and by a posterior displacement
333 of the superior insertion of the pectoral fin (landmark 10). Specimens of La.I., (highest KE; Fig. 6), had a
334 more elongated shape in the head region as well as in the caudal one (Fig. 4, 5; Table I). Overall, the
335 change was constituted by an anterior constriction and elongation of the head (landmarks 1 and 2,
336 semilandmarks from 12 to 15) and by a forward displacement of anterior insertion of second dorsal fin
337 (landmarks 4) and a backward movement of the dorsal side of the caudal peduncle at the insertion of
338 the caudal fin (landmarks 6).

339 **Relationship between morphological differences and geographic separation**

340 The linear regression analysis of the Euclidean distance between the averaged RW1 scores of each
341 locality against the spatial separation index between the sample locations revealed an increasing
342 divergence of morphological pattern with the increase of the S_{db} ($\log(\text{RW1 divergence}) = -$
343 $8.02 + 0.35 * \log(S_{db})$) (Fig. 7a). The 95 % interval of the bootstrapped estimates of the regression slope
344 indicated that it differed from 0 (Fig. 7a). The smallest divergences were in fact observed when localities
345 within a pair held a small separation index (i.e. Le.I. and S.W.S.; Fig. 7a), whereas the highest
346 morphological differences were observed between La.I., on the African shelf, and the three localities of
347 the Sicilian shelf.

348 **Morphospace patterning and relationship with seabed rugosity, a proxy of habitat complexity**

349 Point pattern analysis of the arrangement of samples within the morphospace revealed a clumped
350 pattern for Le.I. (SIm 0.504) and La.I. (SIm 0.509) significantly different from a random arrangement
351 (Table II). No significant differences from a random pattern distribution were observed for S.E.S. (SIm -
352 0.05) and S.W.S. (SIm 0.35) respectively (Table II). Seabed rugosity was lower in S.E.S. and S.W.S. while

353 the highest value was observed in Le.I. (Fig. 7 b). Linear regression analysis of the relationships between
354 the distribution pattern in the morphospace and seabed rugosity showed that the SI_m was positively
355 related to the rise of topographical heterogeneity and roughness of the seafloor ($SI_m = 0.59$
356 $+0.1 \cdot \log(\text{Rugosity})$). The 95 % interval of the slope bootstrapped estimates indicated that it differed
357 from 0 (Fig. 7b).

358 Discussion

359 The identification of phenotypic differences among populations within a geographical area, although
360 not providing evidence of genetic isolation, can indicate the existence of biological groups stably
361 inhabiting distinct environments and for which, immigration and emigration could have limited effects
362 in determining population attributes such as reproduction, longevity or size structure (e.g. Cadrin, 2010;
363 Sequeira *et al.*, 2011). The identification of these units is a crucial requisite for fisheries management
364 because each of these can have unique demographic properties or rebuilding capabilities when faced
365 with exploitation.

366 In our study, intraspecific variation in body morphology of red mullet was found in the Strait of Sicily.
367 The results of the multivariate linear models suggested in fact that red mullet coming from La.I. and
368 S.E.S. differed between each other and with specimens coming from the north western part of the SoS
369 (S.W.S and Le. I.). Specimens of the S.E.S. had a more rounded and higher head profile and posterior
370 displacement of the superior insertion of the pectoral fin. Whereas specimens from La.I. showed an
371 anterior constriction and elongation of the head and a backward movement of the dorsal side of the
372 caudal peduncle. Because of the high number of biotic and abiotic factors [e.g. food availability, feeding
373 conditions, current velocity, habitat complexity, (Langerhans et al., 2003, 2007 Bruckerhoff &
374 Magoulick, 2017, Bracciali et al. 2016; Costa & Cautadella, 2007)] that can potentially induce
375 morphological variability among populations (e.g. Cadrin, 2000), it may be difficult to assess one
376 independently without the effects of the remaining factors. However, in this study the observed
377 morphologic differences seem to fit well the predictions, widely suggested in literature, of effects of
378 hydrodynamic regimes on body shape, consisting in a relatively more streamlined body across
379 increasing gradients of water velocity (e.g. Langerhans et al., 2003, 2007; Sidlauskas et al., 2006;
380 Langerhans & Reznick, 2010; Ellerby & Gerry, 2011; Foster et al., 2015; Bruckerhoff & Magoulick, 2017,
381 Bracciali et al. 2016). The more streamlined body form (i.e. elongation of the head and more developed

382 dorsal size of caudal peduncle), in fact, has been observed in La.I. where the hydrodynamic regime was
383 stronger (Fig. 6). This tapering can be explained as the consequence of an adaptive behavior adopted
384 for a better control of perturbations coming from the surrounding environment [e.g. increase of
385 viscosity of high velocity waters (Sfakiotakis et al. 1999)] due to the water flux (e.g. Webb, 2002; Bracciali
386 et al. 2016) or for maximize thrust while minimizing energy losses when counteracting the action of
387 currents velocity (e.g. Langerhans & Reznick, 2010; Bruckerhoff & Magoulick, 2017).

388 We also found high morphological similarity when localities were low geographically separated (i.e.
389 S.W.S. and Le.I.) and that divergence tended to increase with this geographical separation between
390 localities rised. One crucial concept in evolutionary ecology and biology is that divergent regimes, such
391 as the presence of alternative environments, often induce phenotypic diversification (e.g. Schluter,
392 2000). However, populations mixing (e.g. through passive transport of larvae and active movement of
393 juveniles and adults, gene flow) may promote a reduction of the degree of the induced diversification
394 of phenotypes (e.g. Lenormard, 2002). Ideally, in order to evaluate the relationship between divergence
395 and mixing, the adaptive diversification should be related to a quantitative estimation of the population
396 mixing. In practice, such quantities are difficult to obtain. One good surrogate for the rate of mixing is
397 the geographical distance between populations, as shown by studies focused on its role in determining
398 genetic and phenotypic isolation (e.g. Planes & Fauvelot, 2002; Medina et al. 2007). In our results, for
399 the two close locations situated in the north-western SoS, S.W.S. and Le.I., the population's exchanges
400 favored by their geographical proximity seem to constrain enough the morphological divergence,
401 contrarily to what happened for the other two areas, S.E.S and La.I. These latters, in fact, showed
402 greater morphological differences from the other samples and between each other. It is worth noting
403 that the morphometric distinctness detected among the north-western part of the SoS (S.W.S. and Le.I.)
404 and the two sampling areas situated easterly (S.E.S.) and south-easterly (La.I.) accords with the recent
405 results reported by Gargano *et al.* (2017). These authors described, in fact, a low level of connectivity
406 among populations inhabiting the Sicilian coast and between these and the ones inhabiting the African
407 shelf. In their study, they remarked the existence of physical barriers generated by the interactions
408 between the bottom topography and the currents pattern of the SoS which may limit the latitudinal
409 exchanges of red mullet early life stages within the area (Gargano *et al.*, 2017). Similar outcomes
410 describing a decrease in phenotypic similarities with the geographical distances among the sample
411 location have been found for the red mullet populations in the Aegean Sea (Mamuris *et al.*, 1998).

412 Geographical distance based morphometric divergences at relatively low spatial scale were found also
413 for other species. For instance, in the Cape Verde archipelago system, Medina *et al.* (2008) found a
414 relationship between physical isolation and morphological divergence for the serranid fish
415 *Cephalopholis taeniops* (Valenciennes, 1828). Furthermore, Langerhans *et al.* (2003) showed that the
416 magnitude of morphological divergences tended to increase with increasing spatial distances in two
417 tropical fish species.

418

419 It is recognized that the nature of the shape differences between different geographical populations
420 could be attributed to the different environmental conditions prevailing in each geographical area
421 and/or different genetic structures (Cadrin, 2010). Unfortunately, we do not know whether the
422 morphological diversity of red mullet observed in this study is genetically determined or based on
423 phenotypic plasticity, being both important factors in the evolution of resource polymorphism (e.g.
424 Schluter, 2000). However, based on the present results, it seems that low geographical separation (high
425 population mixing, Le.I.-S.W.S) might reduce the red mullet diversification, regardless of the source of
426 divergence (i.e. genetic or plasticity). The morphological heterogeneity found within the Strait of Sicily
427 provides additional support to previous results obtained by using microsatellite analysis which showed
428 that red mullet cannot be considered a single homogeneous population within the SoS, but rather a
429 pool of units which are locally and partially separated (Maggio *et al.*, 2009). Our study together with
430 these findings suggest that further investigations will need to be implemented to understand the
431 underlying mechanisms behind the differences encountered.

432 While the comparison among shapes gave information on the red mullet populations structure, the
433 morphospace pattern analysis allowed to account for the variability of the different body shapes within
434 each sample location and consequently to speculate about the likely environmental drivers triggering
435 it. Specifically, in this study, the pattern of morphospace occupation indicated that the shape
436 distribution within the morphospace for Le.I. and La.I. showed some degree of grouped allocation.
437 These localities were characterized by a high rugosity index (a proxy of the habitat structural
438 complexity) and by a compacted mud bottom type (Di Lorenzo *et al.*, 2017). Contrarily, in S.W.S. and
439 S.E.S, where the shape distributions were randomly distributed within the morphospace, the rugosity
440 index was lower and sea bottom type was terrigenous mud. It has been suggested that habitat
441 complexity (here seabed rugosity) may drive phenotype expression in order to allow specific characters

442 improving foraging ability and consequently having effects on fitness (e.g. Garduno-Paz *et al.*, 2010).
443 *Mullus barbatus* is a carnivorous fish that scoops up the substratum to detect preys and feed on a wide
444 range of benthic invertebrates, mainly polychaetes but also crustaceans and molluscs (e.g. Bautista-
445 Vega *et al.* 2008). A recent study highlighted the opportunistic feeding behavior of this species, which
446 can change its diet based on the composition of the benthic community and to variations in food item
447 abundance levels and spatio-temporal distribution (Esposito *et al.*, 2013). So, the random distribution
448 of body shape observed in S.W.S. and S.E.S. could be due to the variation of the preys' availability and
449 distribution in response to the low complex and terrigenous mud nature of the sea bottom, which is
450 highly subjected to variation generated by the water mass flowing above it (e.g. Peres and Picard, 1964).
451 Although ours is a speculation, studies have shown that within a population, each individual can use
452 only a part of the population's resources based on their availability (Individual specialization, Bolnick *et al.*
453 2003) leading to changes in foraging behaviour with a resulting rise of morphological variations
454 within a population (Svanbäck and Bolnick, 2007).

455 In addition, the effect of the habitat complexity is strictly linked with several ecological components
456 including multiple inter and intraspecific relationships. The increase of habitat complexity, in fact, acts
457 on the fish biodiversity (García-Charton & Pérez-Ruzafa, 2001; Coll *et al.*, 2010; Danovaro *et al.*, 2010;
458 Farré *et al.* 2015) having as a consequence the rise of both direct biotic interactions (e.g. predation and
459 competition) and indirect effects [apparent competition, facilitation, cascading effects, tri-trophic-level
460 interactions, higher-order interactions (e.g. K Dodds & A Nelson, 2006; Morales-Castilla *et al.*, 2015)].
461 The intensification of interspecific biotic interactions promoted by the rise of habitat complexity in a
462 specific area can lead to increase of morpho-functional differentiations and the rise of species
463 morphological traits specialization (e.g. Montaña *et al.*, 2014, Farre *et al.* 2015). Consequently, a single
464 species (as in our study) or a group of species (e.g. Farré *et al.*, 2015) can develop peculiar solutions for
465 essential functions including foraging efficiency, species coexistences or predation rates (Willis *et al.*,
466 2005; Farré *et al.*, 2015). So, our results suggested that in more complex habitats (Le.I. and La.I.) the
467 red-mullet morphological shapes were clustered, indicating ecological similarity between conspecifics.
468 These results are in agreement with those of Willis *et al.* (2005) who found, considering groups of
469 species, a compression of the niche dimension and an increase of segregation of the morphological
470 traits within the morphospace when the complexity of the habitat rise. This reduction and segregation
471 of the morphological variability for a population within an assemblage may allow to the individuals to

472 exploit those food resources and microhabitats for which its morphology and physiology confer a
473 competitive advantage (Willis *et al.*, 2005; Olsson *et al.*, 2007; Garduno-Paz *et al.*, 2010).

474 In conclusion, the body shape differences between local populations of red mullet in the SoS are
475 consistent with patterns of morphometric diversification, reported in the literature for other fish
476 species, which may reflect optimizations in swimming and feeding performance. Although these
477 differences are in accordance with the different environments considered, it is not clear whether they
478 result from an environmental or genetic basis. Theoretically, both sources of diversification can act
479 simultaneously and may have played a role in the diversification of the red mullet in the SoS. The
480 observed phenotypic variability in relation to the spatial distribution of the red mullet in the study area
481 supports the hypothesis that there are some restrictions to the individuals' dispersal flow among the
482 investigated population units, being more probable the connectivity between areas that are closer
483 between them. By using a morphological approach, in fact, our results integrate the knowledge on the
484 existence of self-recruiting populations of red mullet proposed by Gargano *et al.* (2017), on the basis of
485 larval dispersal between the main spawning and nursery areas in the SoS, thus improving our
486 perception of stock dynamics in terms of adult migration.

487 Geometric morphometric analyses offer the possibility to investigate in a more specific and correct way
488 the population structure (Cadrin, 2010). Our results suggest that a more accurate approach is needed
489 when declaring populations and subpopulations of certain species in small areas, which are traditionally
490 delineated by the assessment agents (e.g. GFCM) throughout geo-economic or political aspects.
491 Nevertheless, although our findings provide some insights on the spatial structure of the red mullet
492 populations within the stock inhabiting the SoS, to confirm our outcomes and define more effective
493 population units for developing effective fishery management recommendations, further studies
494 considering a more abundant number of specimens (e.g. 50 specimens for locality; Cadrin, 2010)
495 coupled with different sources of information [e.g. genetic analyses, parasites, detailed diet analysis,
496 migration patterns, larval dispersal (Cadrin, 2010)] should be implemented.

497

498

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500

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505

506 **Contributions**

507

508 Q.F. : ideas, data preparation, data generation, data analysis, manuscript preparation

509 G.G. : : ideas, manuscript preparation

510 F.F. : manuscript preparation

511 G.D'A. : manuscript preparation

512 A.T. : data preparation

513 A.Z.: data preparation

514

515

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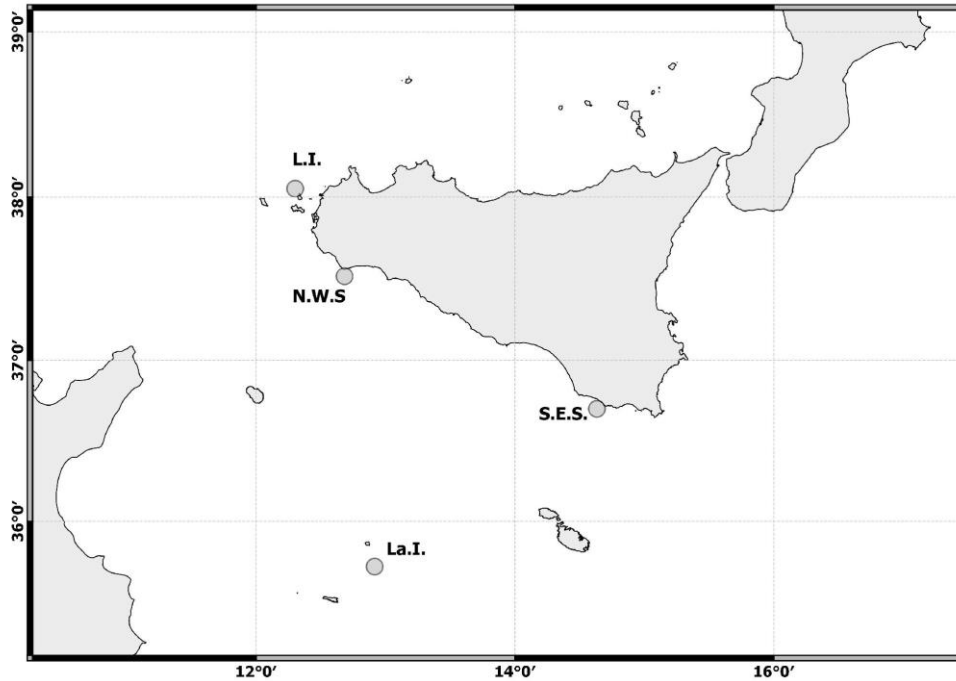
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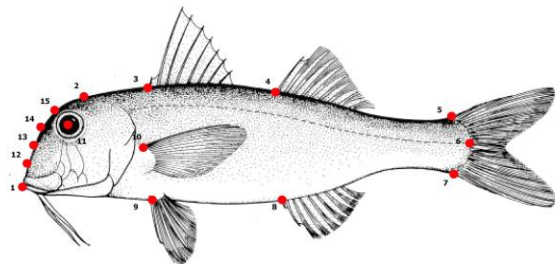
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768 **Figure**

769 **Figure.1 Map of the Strait of Sicily showing the location of the sampling sites: Levanzo Island (Le.I.), South-**
770 **Western-Sicily (S.W.S.), South-Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.).**

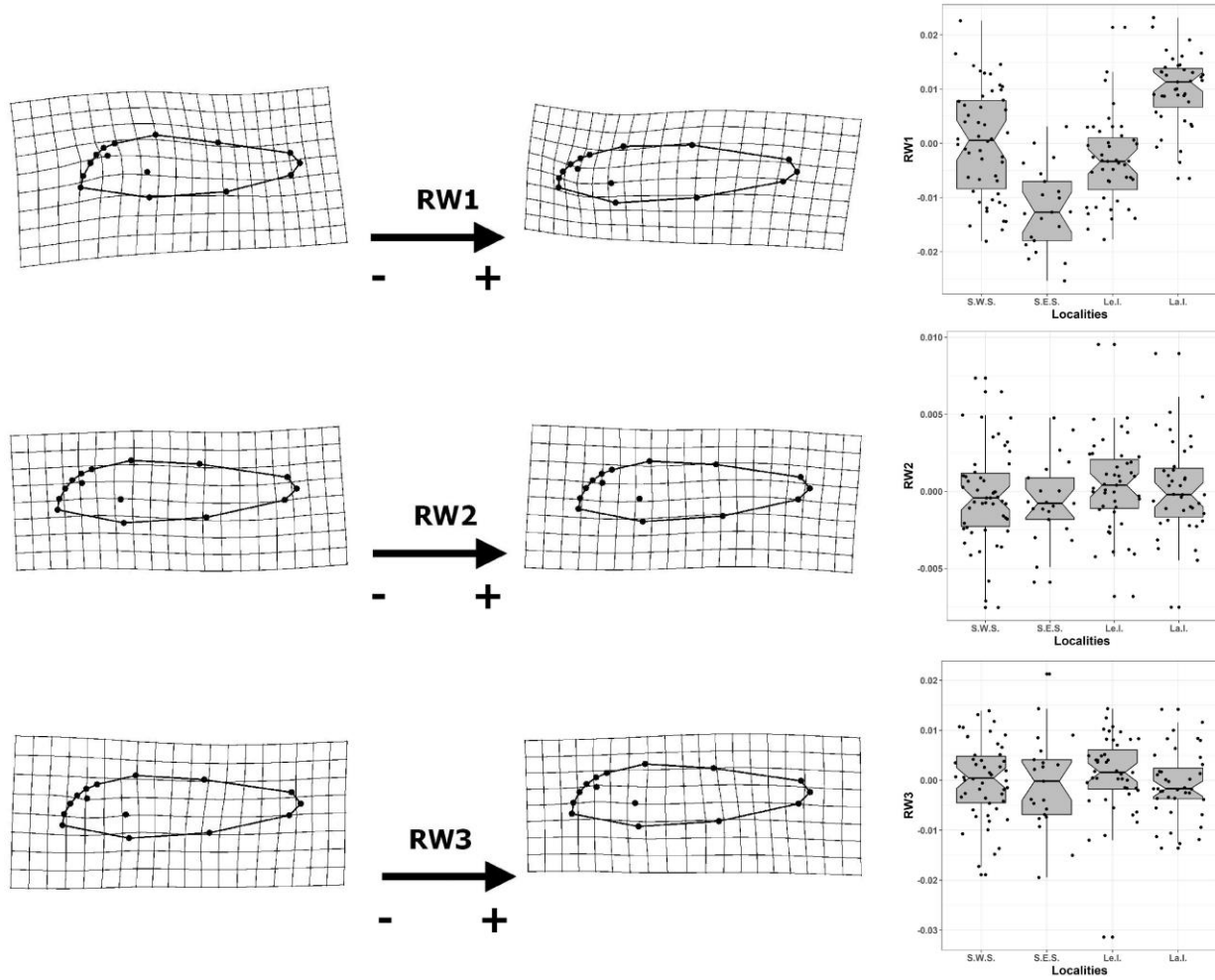


771 **Figure 2. Position of landmarks (1-11) and sliding semilandmarks (12-15) used in morphometric analysis of red**
772 **mullet. (1) tip of snout, (2) head above the extreme part of the eye, (3) anterior insertion of first dorsal fin, (4)**
773 **anterior insertion of second dorsal fin, (5) dorsal side of the caudal peduncle at the insertion of the caudal fin,**
774 **(6) points of maximum curvature of the peduncle, (7) ventral side of the caudal peduncle at the insertion of**
775 **the caudal fin, (8) anterior insertion of the anal fin, (9) anterior insertion of the pelvic fin, (10) superior insertion**
776 **of the pectoral fin, (11) center of the eye (e.g. Cautadella et al. 2007 and Fruciano et al. 2011). (12 to 15)**
777 **semilandmarks chosen to capture the overall shape of the anterior part of the head.**
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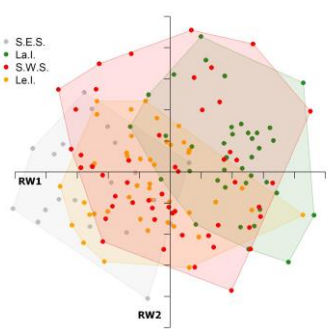


779 **Figure -3. A grid line deformation along each of the three relative warps (RW1, RW2, RW3) is shown to describe**
780 **deformation along these axes of one extreme relative to the other. Box plots of the relative warp scores are**
781 **also reported for each locality. Levanzo Island (Le.I.), South-Western-Sicily (S.W.S.), South-Eastern-Sicily**
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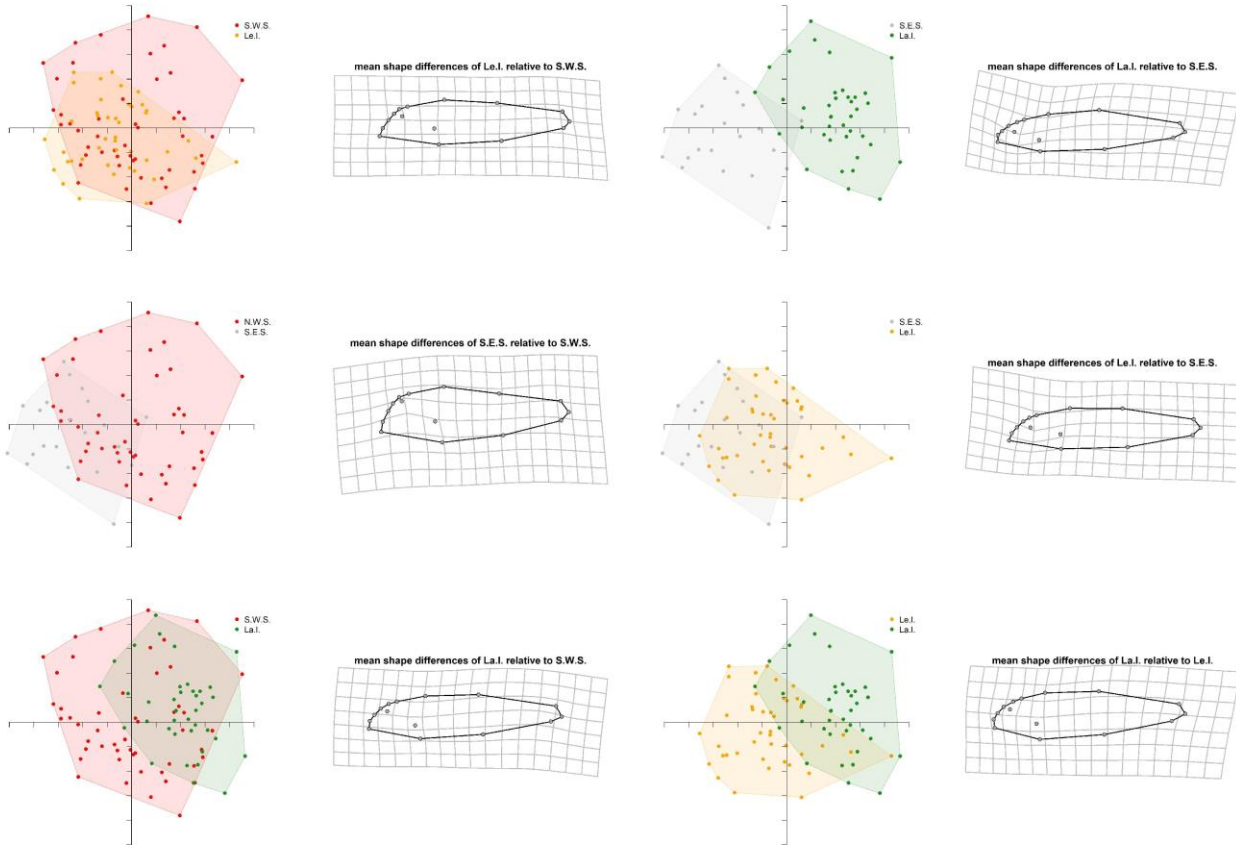
783 (S.E.S.), Lampedusa Island (La.I.). The box represents the data from the first quartile to the third quartile. The
 784 lines indicate the range of data variation (min to max values) excluding the outliers which are the points above and
 785 and below the extreme of the lines (more than 3/2 of the upper and less than 3/2 of the lower quartile). The
 786 segments inside the boxes indicate the median value.



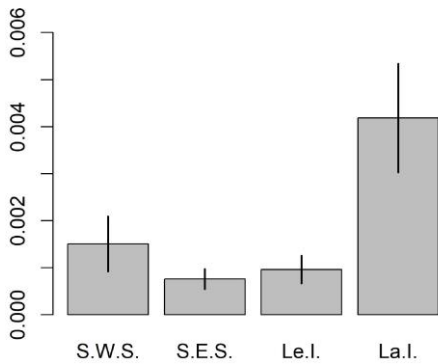
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 789 **Figure 4. Relative warp analysis on overall shape data. Morphospace in which only the first two components**
 790 **(horizontal axis=RW1 and vertical axis=RW2) are shown, explaining respectively 27.2 % and 15.5 % of the**
 791 **overall shape variation of red mullet individuals. Levanzo Island (Le.I.), South-Western-Sicily (S.W.S.), South-**
 792 **Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.).**



794 **Figure 5. Pairwise comparison of the convex hulls delineating the morphospace (Horizontal axis=RW1, Vertical axis=RW2) occupation by red mullet in each locality. For each pair, a deformation grid of the mean shape of one location relative to the other is also shown..** Levanzo Island (Le.I.), South-Western-Sicily (S.W.S.), South-
 795 Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.).
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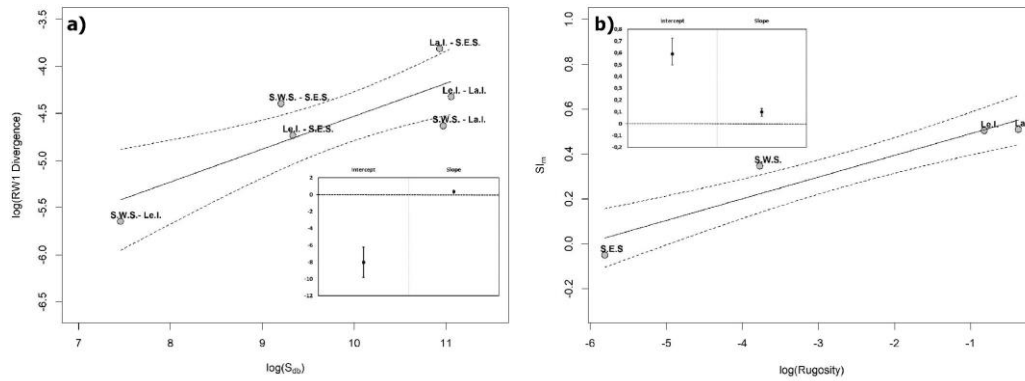
798 **Figure 6. Averaged Kinetic energy (monthly values from 2008 to 2013) and standard deviation of each locality**
 799 **at 40 m (S.W.S. and S.E.S) and 90 m (Le.I. and La.I.) depth. Levanzo Island (Le.I.), South-Western-Sicily (S.W.S.),**
 800 **South-Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.).**
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807 Figure 7. a) Relationship and the +/- 2 bootstrap standard errors between the divergence in RW1 and the
 808 geographical separation (S_{db}) between sample locations. The box at the bottom indicate the 95% of the
 809 bootstrap estimates of both intercept and slope b) Relationship and the +/- 2 bootstrap standard errors
 810 between the Standardized Morista index (SMI) and seabed rugosity (R) characterizing the sample locations.
 811 The box at the bottom indicate the 95% of the bootstrap estimates of both intercept and slope. Levanzo Island
 812 (Le.I.), South-Western-Sicily (S.W.S.), South-Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.).

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TABLES

818 Table 1. Pairwise differences between shapes tested by multivariate linear model using bootstrap resampling
 819 of residuals (n=999). Overall test and univariate test for each relative warp (RW1, RW2, RW3). LR = likelihood
 820 ratio statistic; p=p-value. Levanzo Island (Le.I.), South-Western-Sicily (S.W.S.), South-Eastern-Sicily (S.E.S.),
 821 Lampedusa Island (La.I.).

822

	Overall test for each pairwise comparison			Univariate Test					
	Res.Df	(LR)	p	RW1		RW2		RW3	
				LR	p	LR	p	LR	p
S.W.S. vs Le.I.	91	4.923	0.203	3.46	0.2	0.89	0.59	0.57	0.59
S.W.S. vs La.I.	87	29.64	0.002 **	27.61	0.002	1.76	0.33	0.28	0.60
S.W.S. vs S.E.S.	69	26.04	2e-04 ***	25.55	0.0002	0.46	0.75	0.03	0.86
Le.I. vs La.I.	80	74.49	0.002 **	66.24	0.002	6.68	0.02	1.57	0.22
La.I. vs S.E.S.	58	140.8	0.0198 *	136.86	0.02	3.89	0.13	0.046	0.89
Le.I. vs S.E.S.	62	17.98	0.002 **	17.48	0.002	0.001	0.98	0.49	0.74

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825 Table 2. Morisita and standardizes Moristia Index for each locality. Levanzo Island (Le.I.), South-Western-Sicily
 826 (S.W.S.), South-Eastern-Sicily (S.E.S.), Lampedusa Island (La.I.). imor = unstandardized Morisita index; mclu =
 827 clumpedeness index muni=uniform index; SI_m =Standardized Morisita index. Imor,mclu and muni are reported
 828 because used to calculate SI_m

829

	imor	mclu	muni	SI_m	p
Le.I.	2,34	1,67	0,42	0,50	2E-04
S.W.S.	1,41	1,59	0,49	0,35	8E-02
La.I.	3,35	1,74	0,36	0,51	7E-08
S.E.S.	0,88	2,41	-0,22	-0,05	6E-01

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