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Corresponding Author	Family Name	Arechavala-Lopez
	Particle	
	Given Name	P.
	Suffix	
	Division	Department of Marine Sciences and Applied Biology
	Organization	University of Alicante
	Address	P.O.Box 99, Alicante, 03080, Spain
	Email	pablo.arechavala@ua.es

Author	Family Name	Sanchez-Jerez
	Particle	
	Given Name	P.
	Suffix	
	Division	Department of Marine Sciences and Applied Biology
	Organization	University of Alicante
	Address	P.O.Box 99, Alicante, 03080, Spain
	Email	

Author	Family Name	Bayle-Sempere
	Particle	
	Given Name	J. T.
	Suffix	
	Division	Department of Marine Sciences and Applied Biology
	Organization	University of Alicante
	Address	P.O.Box 99, Alicante, 03080, Spain
	Email	

Author	Family Name	Sfakianakis
	Particle	
	Given Name	D. G.
	Suffix	
	Division	Biology Department
	Organization	University of Crete
	Address	P.O. Box 1470, Iraklion, Crete, 71110, Greece
	Email	

Author	Family Name	Somarakis
	Particle	
	Given Name	S.
	Suffix	
	Division	Hellenic Centre for Marine Research

Organization	Institute of Marine Biological Resources
Address	P.O. Box 2214, Iraklion, Crete, 71003, Greece
Email	

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Abstract Gilthead seabream (*Sparus aurata* L.) and European seabass (*Dicentrarchus labrax* L.) are important commercial marine fish species both for aquaculture and fisheries in the Mediterranean. It is known that farmed individuals escape from farm facilities, but the extent of escape events is not easy to report and estimate because of the difficulty to distinguish between wild and farmed individuals. In this study, significant differences evidence that the cranial and body regions of seabream and seabass are different regarding their farm or wild origin at a different scales are provided through morphometry. Morphological variations have been shown to be a valuable tool for describing changes in shape features. Therefore, the biomass contribution of escapees to local habitats could be determined by identifying escaped individuals from fisheries landings as a first step to assess the potential negative effects of fish farm escapees on the environment, and their influence on wild stocks and local fisheries.

Keywords (separated by '-') Seabream - Seabass - Morphometry - Escapees - Aquaculture - Fisheries landings

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2 **Morphological differences between wild and farmed**
3 **Mediterranean fish**

4 **P. Arechavala-Lopez · P. Sanchez-Jerez ·**
5 **J. T. Bayle-Sempere · D. G. Sfakianakis ·**
6 **S. Somarakis**

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Keywords Seabream · Seabass · Morphometry · 29
Escapees · Aquaculture · Fisheries landings 30

Introduction 32

Gilthead seabream (*Sparus aurata* L., Fam. Sparidae) 33
and European seabass (*Dicentrarchus labrax* L., Fam. 34
Moronidae) are the important commercial marine fish 35
species along the Mediterranean and Eastern Atlantic 36
coastline both for aquaculture and fisheries. In 2008, 37
the total aquaculture production of seabream and 38
seabass in Europe were 89,354 and 58,467 t, respec- 39
tively, and total landings reached 7,812 and 8,528 t, 40
respectively (FAO, 2011). It is well known that reared 41
individuals escape from farm facilities due to technical 42
and operational failures (Dempster et al., 2007), but 43
the knowledge concerning ecological and genetic 44
impacts of these escapees on the Mediterranean 45
ecosystem is still sparse. Escaped fish could be present 46
on spawning areas and could interbreed with native 47
populations as was found for salmonids (Naylor et al., 48
2005) and for cod (Uglen et al., 2008; Meager et al., 49
2009). Furthermore, it has been reported that escaped 50
seabream and seabass were able to swim away from 51

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A2 P. Arechavala-Lopez (✉) · P. Sanchez-Jerez ·
A3 J. T. Bayle-Sempere
A4 Department of Marine Sciences and Applied Biology,
A5 University of Alicante, P.O.Box 99, 03080 Alicante,
A6 Spain
A7 e-mail: pablo.arechavala@ua.es

A8 D. G. Sfakianakis
A9 Biology Department, University of Crete,
A10 P.O. Box 1470, 71110 Iraklion, Crete, Greece

A11 S. Somarakis
A12 Hellenic Centre for Marine Research, Institute of Marine
A13 Biological Resources, P.O. Box 2214, 71003 Iraklion,
A14 Crete, Greece

52 farm facilities to nearby farms, local fishing grounds
53 and coastal habitats (González-Lorenzo et al., 2005;
54 Toledo-Guedes et al., 2009; Arechavala-Lopez et al.,
55 2011a, b). Thus, the potential for negative ecological
56 consequences to occur through predation, competition
57 or transmitting pathogens to closely reared and wild
58 populations is significant (Dempster et al., 2007). For
59 a better understanding of these potential negative
60 effects, it is important to quantify the number of
61 individuals that escape from sea-cages, and to analyse
62 their mobility, spatial distribution and survival.
63 Because of the difficulty in surveying directly the
64 escapes events, it is imperative to distinguish escapees
65 from native individuals in the natural habitats as a first
66 step to assess their contribution to fisheries landings,
67 for instance examining captured individuals from
68 local fisheries after an escape event.

69 In hatcheries, fish grow faster and frequently with
70 different patterns and environment than in the wild and
71 this phenomenon has been mainly utilized to distinguish
72 between wild and reared salmonids with a relatively
73 high degree of certainty (Swaine et al., 1991; Fleming
74 et al., 1994; Hard et al., 2000; Fiske et al., 2005).
75 Differential relative growth of body parts conditioned
76 by environmental factors is a common feature of fish
77 development (Osse, 1990; Osse & van den Boogaart,
78 1995, 1999; Gisbert, 1999; Loy et al., 2001). In several
79 species, developmental modifications may be closely
80 linked also to ontogenetic changes in resource use
81 (Webb & Weihs, 1986; Hernandez & Motta, 1997;
82 Sagnes et al., 1997; Ward-Campbell & Beamish, 2005).
83 Such different developmental modifications may exist
84 between wild and farmed fish given that they experience
85 large differences in feeding regimen and environment.
86 Moreover, in reared seabream and seabass, the presence

of malformations or morphoanatomical anomalies has
been widely documented (Paperna, 1978; Francescon
et al., 1988; Balebona et al., 1993; Boglione et al., 1993,
2001; Marino et al., 1993; Chatain, 1994; Koumoun-
dourous et al., 1997; Loy et al., 1999, 2000; Afonso
et al., 2000; Sfakianakis et al., 2006). The objective of
this study is to assess the body measures which can
discriminate between farmed or wild origin of seabream
and seabass in the Mediterranean Sea, and therefore, if
the existence of some specific measurements could be
applied to study the contribution on wild populations
and fisheries landings.

Materials and methods

Sampling and morphometric measurements

A total of 200 wild seabream and 200 farmed seabream
individuals, as well as 200 wild seabass and 200 farmed
seabass individuals, from Spain and Greece, were used
in this study (Table 1). Wild and farmed fish from
Spain were obtained during the period of July 2009–
June 2010, from two different localities and farms,
respectively (Fig. 1). Fish from Greece were obtained
in October 2009, from a single locality and a single
farm (Fig. 1). Each seabream or seabass was photo-
graphed with a digital camera (Canon® Powershot-
G10) mounted on tripod with a light source. A ruler was
used on each photograph to ensure correct calibration
in the following image processing. Morphological
landmarks were selected to give a precise definition
of the fish morphology (Humphries et al., 1981; Strauss
& Bookstein, 1982). Altogether 16 morphological
landmarks on seabream (Fig. 2; Table 2) and 17

Table 1 Characteristics of seabream and seabass specimens used in this study

Species	Locality	Origin	n	Ls (cm)		Wt (g)	
				Range	Mean ± SD	Range	Mean ± SD
<i>Sparus aurata</i>	Spain	Wild	100	25.3–47.9	34.6 ± 4.8	393–2628	1077 ± 445
		Farmed	100	28.6–39.4	30.7 ± 1.2	796–1105	906 ± 53
	Greece	Wild	100	13.1–17.5	15.3 ± 1.1	47–103	71 ± 1
		Farmed	100	13.0–18.7	15.6 ± 1.1	40–132	79 ± 1
<i>Dicentrarchus labrax</i>	Spain	Wild	100	17.3–54.7	31.1 ± 10.1	84–2920	671 ± 676
		Farmed	100	32.7–38.6	35.6 ± 1.1	821–1075	918 ± 48
	Greece	Wild	100	18.1–27.8	23.6 ± 1.9	80–280	152 ± 37
		Farmed	100	19.0–25.0	21.4 ± 1.2	91–197	128 ± 22

Fig. 1 Maps of the study areas in Spain and Greece, showing the wild and farmed fish sampling localities

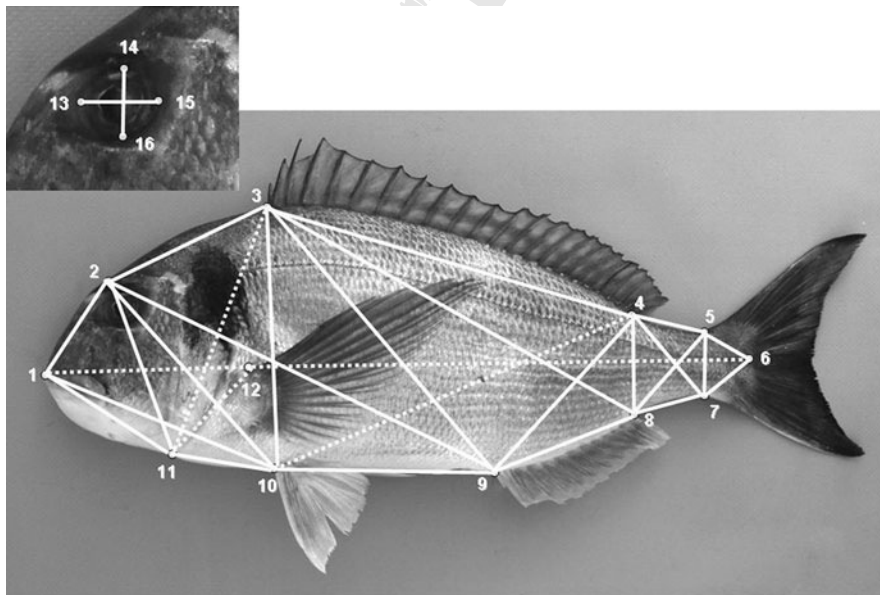
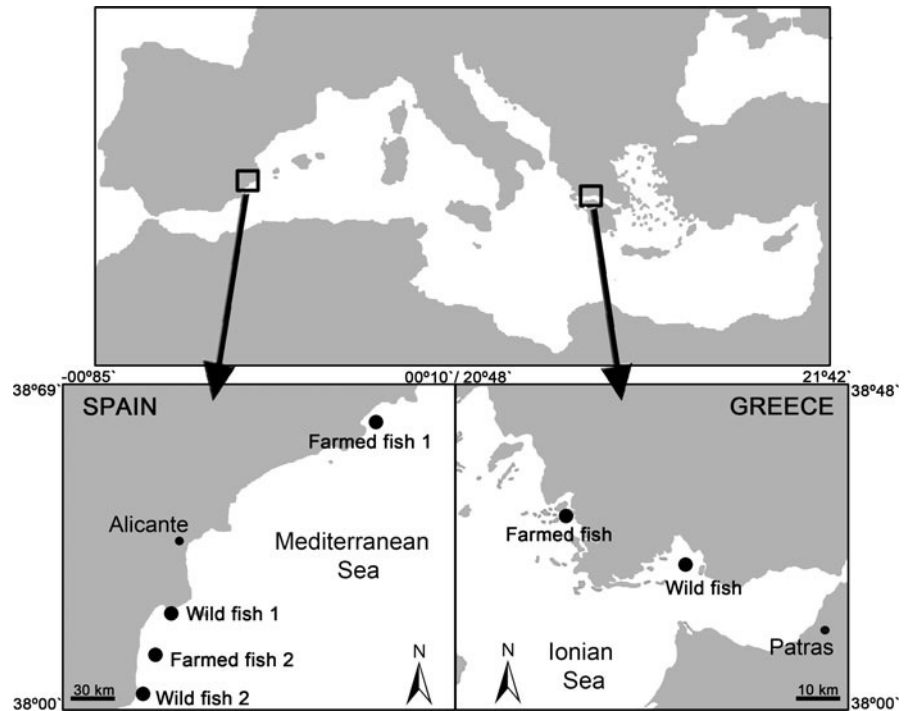


Fig. 2 The 16 landmarks and the distances measured which were used for the morphological analysis on seabream. The morphometric traits described from the landmarks are shown in Table 2. *Solid lines* TNS; *dotted line* additional measurements. 1 tip of the premaxillary; 2 point of maximum curvature in the head profile curve; 3 anterior insertion of dorsal fin; 4 posterior insertion of dorsal fin; 5 dorsal point at least depth of caudal

peduncle; 6 posterior extremity of the lateral line; 7 ventral point at least depth of caudal peduncle; 8 posterior insertion of anal fin; 9 anterior insertion of anal fin; 10 anterior insertion of pelvic fin; 11 insertion of the operculum on the profile; 12 dorsal insertion of pectoral fin; 13 most anterior point of the eye; 14 most dorsal point of the eye; 15 most posterior point of the eye; 16 most ventral point of the eye

118 morphological landmarks on seabass were used
 119 (Fig. 3; Table 2), and they were placed using the
 120 image processing programme ImageJ (Abramoff et al.,
 121 2004), in which the morphological landmarks are given
 122 as x and y co-ordinates. This tool is called Truss
 123 Network System (TNS) and covers the entire fish in a
 124 uniform network which should theoretically increase
 125 the likelihood of extracting morphometric differences
 126 within and between species. A regionally unbiased
 127 network of morphometric measurements over the
 128 two-dimensional outline of a fish should give more
 129 information about local body differences than a con-
 130 ventional set of measurements (Bookstein, 1982;
 131 Strauss & Bookstein, 1982). A total of 31 morpho-
 132 logical vectors were selected among the landmarks on
 133 seabream and 30 morphological vectors on seabass
 134 (Table 2). The distances between the landmarks were
 135 determined from their co-ordinates. The repeatability
 136 of all measurements was determined by measuring 20
 137 seabream and 20 seabass from each group three differ-
 138 ent times. The coefficient of variation ranged from
 139 0.5 to 2%, which indicates a high accuracy and
 140 repeatability of this method. Moreover, morphometric
 141 indices such as Fulton's Condition Index [$K = 100 \times$ total
 142 weight/(total length)³], Cephalic Index [CI = (head
 143 length/total length)] and Relative Profile Index [RP =
 144 (maximum body height/total length)] were computed
 145 from linear and weight measurements.

146 Statistical analysis

147 In order to avoid the effect of the different specimen's
 148 lengths in the study, all morphological traits were size
 149 adjusted using the method described by Reist (1985),
 150 because heterogeneity in size among samples pro-
 151 duces heterogeneity in measurements. These transfor-
 152 mations were done separately for the different group
 153 analysis (Spain, Greece and both together Spain-
 154 Greece) to avoid interference from the other groups.
 155 All the size-correlated traits were standardized to a
 156 mean of zero and a standard deviation of 1. Multivar-
 157 iate statistics (SPSS, version 15.0 for Windows) were
 158 used to test for intra- and inter-groups variation.
 159 Statistical differences for size and all the morphomet-
 160 ric indices among groups was tested by ANOVA at
 161 $P < 0.05$. A principal component analysis (PCA),
 162 with varimax rotation was selected because the
 163 rotation minimizes the number of variables that have

Table 2 Morphological traits of seabream and seabass mea-
 sured from the landmarks in Figs. 2 and 3

<i>Sparus aurata</i>		<i>Dicentrarchus labrax</i>	
Code	Landmark	Code	Landmark
A1	1-2	A1	1-2
A2	2-10	A2	2-12
A3	10-11	A3	1-12
A4	1-11	B1	2-3
A5	1-10	B2	3-11
A6	2-11	B3	11-12
B1	2-3	B4	2-11
B2	3-9	B5	3-12
B3	9-10	C1	3-4
B4	2-9	C2	4-10
B5	3-10	C3	10-11
B6	3-11	C4	3-10
C1	3-4	C5	4-11
C2	4-8	D1	4-5
C3	8-9	D2	5-9
C4	3-8	D3	9-10
C5	4-9	D4	4-9
C6	4-10	D5	5-10
D1	4-5	E1	5-6
D2	5-7	E2	6-8
D3	7-8	E3	8-9
D4	4-7	E4	5-8
D5	5-8	E5	6-9
E1	5-6	F1	6-7
E2	6-7	F2	7-8
F1	1-12	F3	1-13
F2	11-12	F4	7-13
F3	6-12	Eye L	14-16
Eye L	13-15	Eye H	15-17
Eye H	14-16	SL	1-7
SL	1-6		

high loadings on a factor. All PCAs with eigen-
 value > 1.00 were considered as important (Chatfield
 & Collins, 1983) and variables were tested by
 ANOVA at $P < 0.05$. Discriminant analyses were
 then used to test for group membership. The different
 discriminant functions are hereafter described as DC1,
 DC2, etc. ANOVA was used to test if there were
 differences in morphological traits between the wild
 and the farmed seabream and seabass, respectively.

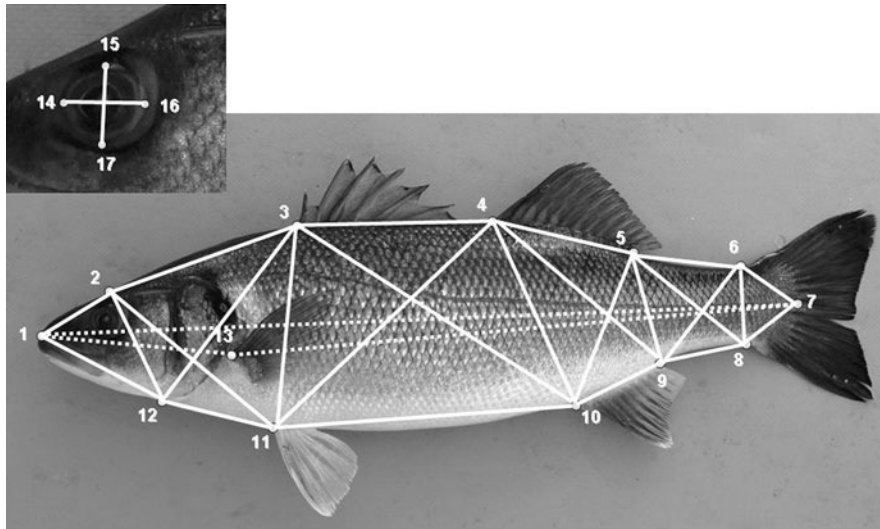


Fig. 3 The 17 landmarks and the distance measured which were used for morphological measurement on seabass. The morphological traits described from the landmarks are shown in Table 2. *Solid lines* TNS; *dotted line* additional length measures. 1 tip of the premaxillary; 2 point of maximum curvature in the head profile curve; 3 anterior insertion of the first dorsal fin; 4 anterior insertion of the second dorsal fin; 5 posterior insertion of the second dorsal fin; 6 dorsal point at least

depth of caudal peduncle; 7 posterior extremity of the lateral line; 8 ventral point at least depth of caudal peduncle; 9 posterior insertion of anal fin; 10 anterior insertion of anal fin; 11 anterior insertion of pelvic fin; 12 insertion of the operculum on the profile; 13 dorsal insertion of pectoral fin; 14 most anterior point of the eye; 15 most dorsal point of the eye; 16 most posterior point of the eye; 17 most ventral point of the eye

173 Results

174 Results evidenced clear differences between wild and
 175 farmed fish, mainly on the cranial and body regions,
 176 for both seabass and seabream. It should be noted that
 177 seabream from Spain and Greece were of different
 178 sizes (ANOVA, $P \leq 0.05$), while sizes of seabass
 179 were similar in the two countries (ANOVA, $P > 0.05$;
 180 Table 1). Fulton's Condition Index (K) revealed sig-
 181 nificant differences between wild and farmed individ-
 182 uals of both studied species (ANOVA; $P \leq 0.01$) with
 183 the farmed fish exhibiting the highest values (Fig. 4a,
 184 b). Cephalic Index (CI) values were significantly
 185 different in seabass (ANOVA; $P \leq 0.05$), where wild
 186 fish showed the highest values in both countries
 187 (Fig. 4c); but there were no significant differences
 188 between wild and farmed seabream specimens
 189 (ANOVA; $P > 0.05$; Fig. 4d). However, values of
 190 Relative Profile Index (RP) were significantly differ-
 191 ent among seabream from different origins (ANOVA;
 192 $P \leq 0.05$), where farmed fish showed higher values
 193 than wild fish (Fig. 4e), while there were no differ-
 194 ences for seabass in both countries (ANOVA;
 195 $P > 0.05$; Fig. 4f).

196 A combination of five principal components 196
 197 explained as much as 87.03% of the variation of 197
 198 size-adjusted body morphology variables for Spanish 198
 199 seabream (ANOVA, $P \leq 0.01$; Table 3). In case of 199
 200 Greece, eight principal components explained as much 200
 201 as 85.69% of this variation in seabream, but only four 201
 202 with significant differences (ANOVA, $P \leq 0.01$; 202
 203 Table 3). In both countries, the most important 203
 204 differences were located in the anterior body portion, 204
 205 principally in head measurements and body height. 205
 206 Discriminant analysis presented four differentiated 206
 207 groups in Spain, belonging to the two fish farms and 207
 208 two control localities (Fig. 5). Two percent of wild 208
 209 fish were not adequately assigned, which may indicate 209
 210 a cultivated origin, while 100% of the farmed fish were 210
 211 correctly classified. Since there were only two groups 211
 212 from Greece, the discriminant analysis gave only one 212
 213 function and it was therefore not possible to plot the 213
 214 relationship between components. However, classifi- 214
 215 cation score for the discriminant analysis resulted in 215
 216 98% of wild fish and 99% of farmed fish from Greece 216
 217 being correctly classified. Comparisons between wild 217
 218 and farmed seabream according to their Spanish or 218
 219 Greek origin showed clear differences for almost all of 219

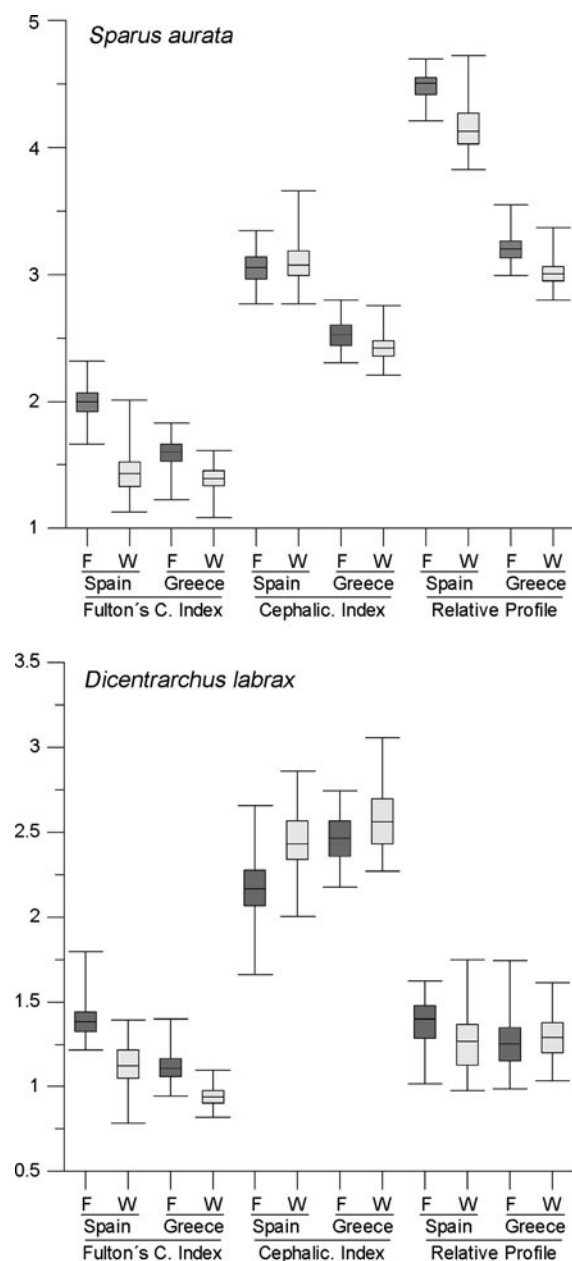


Fig. 4 Morphometric indices of wild and farmed seabream and seabass from Spain and Greece. Bars show mean values \pm standard deviation

220 the taken measurements (Fig. 6). Nonetheless, dis-
 221 criminant analysis exhibited that around 3% of wild
 222 individuals, both from Greece and Spain, could be of
 223 cultivated origin (Table 4). It is remarkable that these
 224 individuals presented high indices values. The differ-
 225 ences in morphological traits were significant
 226 (ANOVA, $P \leq 0.05$) for all discriminant functions

227 for all groups, and could be explained by the group
 228 origin (Wilks' λ , ANOVA, $P \leq 0.01$; Table 5).

229 Significant differences in morphological traits
 230 between wild and farmed seabass in Spain were
 231 described by two components in the PCA analysis
 232 which explained 86.47% of the variation (ANOVA,
 233 $P \leq 0.01$; Table 6). First component principally cor-
 234 related with longitudinal and transversal body mea-
 235 surements, while head and eye measurements were
 236 most representative for the second component. Vari-
 237 ation in morphological traits between farmed and wild
 238 seabass from Greece was explained by five principal
 239 components (80.12%) but only three of them pre-
 240 sented significant differences (ANOVA, $P \leq 0.05$;
 241 Table 6). These differences were mainly due to eye
 242 and head measurements (PC1) and some transversal
 243 body measurements (PC2). Discriminant analysis plot
 244 for Spanish seabass groups illustrated a pronounced
 245 variation in the morphological traits between wild
 246 and farmed fish, where the two locations of farmed fish
 247 were more similar between them, and the two wild
 248 groups were considerably more heterogeneous
 249 (Fig. 7). However, the 2% of wild fish from Spain
 250 were not correctly classified, whereas 100% of the
 251 farmed fish were correctly assigned. Since there were
 252 only two groups of seabass from Greece, the discrim-
 253 inant analysis gave only one function and it was
 254 therefore not possible to plot the relationship between
 255 components. However, discriminant analysis correctly
 256 grouped the 100% of individuals within their respec-
 257 tive group.

258 When comparing morphological traits for seabass
 259 from Spain and Greece together, significant differ-
 260 ences were explained by two principal components
 261 (93.52%; ANOVA, $P \leq 0.01$). Body measurements
 262 were mainly located on the first component (PC1:
 263 56.2%), while head and eyes measurements were more
 264 important in the second one (PC2: 37.2%). The first
 265 two resulting functions from discriminant analysis
 266 explained 70.4 and 23.1% of the variation, respec-
 267 tively (Table 8); and the differences in morphological
 268 traits were significant (Wilks' λ , ANOVA, $P \leq 0.01$;
 269 Table 8). Plotting these two functions, the first one
 270 grouped the seabass according to their geographical
 271 origin, while the second function grouped the samples
 272 according to their wild or farm origin (Fig. 8).
 273 Moreover, 98 and 99% of reared seabass from Spain
 274 and Greece, respectively, were correctly grouped
 275 (Table 7). Furthermore, 88% of wild seabass from

Table 3 Component loadings, percent of variance (% V) and eigenvalues (Eigen.) for the principal components (with varimax rotation) in the Spain and Greece groups analyses for seabream

	Spain					Greece								
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	
Eye H	0.954					A5	0.859							
B2	0.920					A3	0.849							
B5	0.920					A2	0.848							
A6	0.907					B5	0.823							
C4	0.879					B6	0.708							
A5	0.869					D2	0.661							
A4	0.866					F1	0.660							
B6	0.866					F2	0.652							
B4	0.855					B4	0.639							
C6	0.850					A6	0.524							
B3	0.827					B3	0.516							
B1	0.818					C1		0.878						
A2	0.808					C4		0.853						
C5	0.802					B2		0.739						
F1	0.795					B1		-0.557						
C2	0.767					C3			0.897					
D4	0.756					C5			0.830					
E2	0.754					F3			0.614					
D2	0.752					C6			0.574					
D5	0.750					D3				0.868				
C3	0.729					D5			0.844					
D3	0.725					C2			0.525					
D1		-0.661				E2				0.831				
F2		0.477				E1				0.813				
C1			0.628			Eye L					0.850			
A1			0.430			Eye H					0.723			
F3			0.484			A4					0.596			
A3			0.463			D4						0.746		
E1				0.517		D1						0.832		
Eye L					0.594	A1							0.861	
% V	30.03	20.52	16.51	14.81	5.17		24.18	11.12	10.93	10.07	8.93	8.56	6.67	5.22
Eigenv.	9.01	6.15	4.95	4.44	1.55		7.25	3.34	3.28	3.02	2.68	2.57	2.01	1.59
ANOVA	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01	<0.01	0.61	0.16	0.92	<0.01	0.82

Components with significant differences (ANOVA; $P \leq 0.05$) in morphological traits between farmed and wild seabream are marked in bold (see Table 2 for definition of characters)

276 Spain were correctly assigned, but 5 and 7% were
 277 assigned to Greek and Spanish farm origin. Only 1%
 278 of wild seabass from Greece were not correctly
 279 grouped (Table 7).

280 Discussion

281 Body morphology was clearly different between wild
 282 and farmed fish for both species. The spatial

consistency of these results indicate the usefulness of 283
 these indices in discriminating the origin of the studied 284
 species, such as the CI for seabass, the RP for 285
 seabream or Fulton's Condition Index for both 286
 species. In addition, morphometric analyses suggest 287
 that most differences are located primarily in the head 288
 and anterior region of the body of the fish. Specifically, 289
 these differences on seabream were focused either on 290
 the head height (B5) or the distances from the base of 291
 the pectoral fin to the edges of the mouth (A5) and to 292

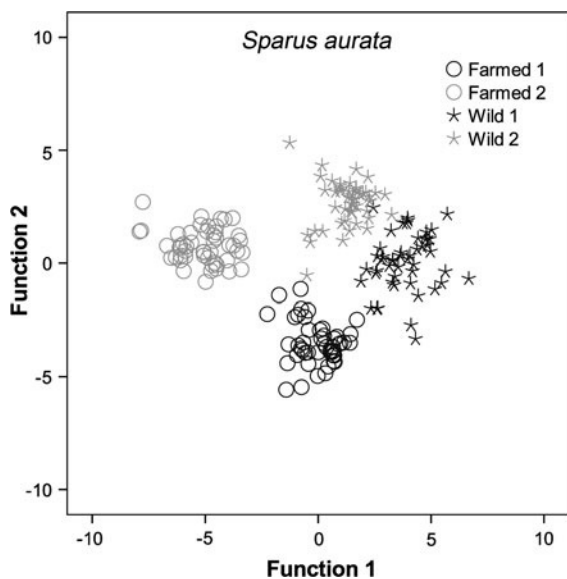


Fig. 5 Scatterplot of functions 1 and 2 for the discriminant analysis at Spain group including the two locations of farmed seabream and the two localities of wild seabream

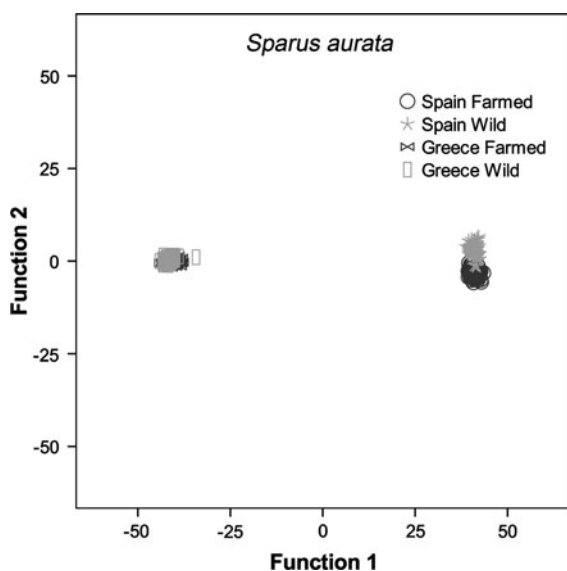


Fig. 6 Scatterplot of functions 1 and 2 for the discriminant analysis including the groups of farmed and wild seabream from Spain and Greece

293 forehead (A2), (see Table 2; Fig. 2); while for seabass
 294 they were on head and body length (F3 and F4,
 295 respectively), proportions of the eye and the distances
 296 from the base of the operculum to the edge of the
 297 mouth (A2) and to the forehead (A3), (see Table 2;
 298 Fig. 3).

299 These morphological differentiations could be due
 300 to either the selective breeding programmes applied
 301 in aquaculture, genetic drift following founding
 302 generations, or the different origin of fish used as
 303 broodstocks (Karaiskou et al., 2009). Although the
 304 accidental escapes of fish from farms have certainly
 305 contributed to a mix of all gilthead seabream genetic
 306 stocks (Sola et al., 2006), a high genetic differentia-
 307 tion between cultivated and wild populations from the
 308 same area has been reported, which might indicate no
 309 evidence for significant genetic flow between them
 310 (Alarcón et al., 2004). The first genetics studies
 311 carried out on gilthead seabream populations reported
 312 conflicting data concerning the existence of panmictic
 313 (Cervelli et al., 1985) or subdivided populations
 314 (Funkenstein et al., 1990). More recent studies have
 315 depicted a picture of species subdivision that still
 316 needs to be clarified. The results of Arabaci et al.
 317 (2010) suggest a slight but significant population
 318 structure for the Mediterranean Sea and Atlantic
 319 Ocean, but not apparently associated with geographic
 320 or oceanographic factors (Alarcón et al., 2004; Ben-
 321 Slimen et al., 2004; De Innocentiis et al., 2004;
 322 Karaiskou et al., 2005; Rossi et al., 2006). In addition
 323 to fish origin, it should be noted that the mean sizes
 324 among Spanish and Greek seabream in this study were
 325 significantly different. Mean size (both length and
 326 weight) of Spanish seabream proved to be higher than
 327 the group from Greece. Thus, some morphological
 328 variations between regions may be the result of
 329 differences in individual's age, and hence body size.
 330 Gilthead seabream are characterized by remarkable
 331 anatomical changes throughout their life history
 332 (Cataldi et al., 1987), and this species is known to
 333 undergo ontogenetic shifts in feeding habits (Mariani
 334 et al., 2002; Tancioni et al., 2003). Furthermore, a
 335 pattern of allometric growth on different body regions
 336 was characterized for each age-stage (Russo et al.,
 337 2007). Further studies will be necessary to compare
 338 different sizes of farmed and wild seabream from
 339 different geographical regions.

340 In the case of European seabass, numerous genetic
 341 population differentiation studies at different geo-
 342 graphic scales have led to the identification of three
 343 genetically distinct zones: the northeastern Atlantic
 344 Ocean, the western Mediterranean and the eastern
 345 Mediterranean (Patarnello et al., 1993; Allegrucci
 346 et al., 1997; García de León et al., 1997; Castilho &
 347 McAndrew, 1998; Sola et al., 1998; Bahri-Sfar et al.,

Table 4 Inter-group classification score result (in %) for the discriminant analysis of the four groups of seabream

<i>Sparus aurata</i>	Group	Spain		Greece		Total
		Farmed	Wild	Farmed	Wild	
Origin group (%)	Spain farmed	100	0	0	0	100
	Spain wild	3	97	0	0	100
	Greece farmed	0	0	98	2	100
	Greece wild	0	0	3	97	100

Table 5 Data from the intra- and inter-groups discrimination analyses

	<i>F</i>	Eigenvalue	Percent variance	Cumulative variance	Canonical correlation	Wilks' λ	χ^2	<i>df</i>	<i>P</i>
Spain	1	10.849	65.6	65.6	0.957	0.009	884.059	60	<0.001
Spain	2	5.132	31.0	96.6	0.915	0.105	421.750	38	<0.001
Spain	3	0.555	3.4	100.0	0.598	0.643	82.608	18	<0.001
Greece	1	4.975	100.0	100.0	0.912	0.167	327.129	30	<0.001
Spain–Greece	1	1695.914	99.5	99.5	1.000	0.000	4073.060	90	<0.001
Spain–Greece	2	5.206	0.3	99.8	0.916	0.040	1232.292	58	<0.001
Spain–Greece	3	3.057	0.2	100.0	0.868	0.247	534.946	28	<0.001

The groups of seabream were classified from these functions (*F*) in the discriminant analysis

348 2000; Castilho & Ciftci, 2005; Ergüden & Turan,
 349 2005; Katsares et al., 2005; Lemaire et al., 2005). In
 350 this study, through discriminate analysis, some wild
 351 seabass from Spain (7%) were grouped along with
 352 farmed fish from Spain, which probably belong to an
 353 escapee group; but some others (5%) were grouped
 354 with seabass from Greek farms. Cases in which
 355 individuals do not cluster with other samples belong-
 356 ing to the same geographical origin are not surprising,
 357 since eggs or fingerlings originating from the western
 358 basin were most likely used to seed many hatcheries
 359 around the Mediterranean when seabass aquaculture,
 360 and therefore, escapes into the wild, began in the early
 361 1980s (Haffray et al., 2006).

362 On the other hand, phenotypic differences are
 363 not necessarily indicative of genetic differentiation
 364 between populations (Ihssen et al., 1981; Allendorf,
 365 1988), and thus the detection of morphological
 366 differences among populations cannot usually be
 367 taken as evidence of genetic differentiation (Turan,
 368 1999). Phenotypic plasticity of fish allows them to
 369 respond adaptively to environmental change by mod-
 370 ification to their physiology and behaviour which

371 leads to changes in their morphology, reproduction or
 372 survival that mitigate the effects of environmental
 373 variation (Stearns, 1983; Meyer, 1987). Unlike wild
 374 populations, farmed fish live inside the cages with a
 375 periodic feeding rate and easily available food,
 376 suggesting that foraging is different from wild fish
 377 (Arabaci et al., 2010). Therefore, the morphological
 378 differences found in this study between wild and
 379 farmed seabream agree with those differences found
 380 by Grigorakis et al. (2002) where wild seabream
 381 presented lower body height, sharper snout and more
 382 spindle-shaped body than cultured seabream. More-
 383 over, such differences could be partly explained by
 384 dietary shifts, which induce changes on the body
 385 shape (Keast, 1978), influencing prey selection
 386 and catch efficiency (Mérigoux & Ponton, 1998).
 387 Furthermore, as they have been widely reported for
 388 farmed seabream and seabass, all fish species may
 389 develop shape abnormalities under farming conditions
 390 (Divanach et al., 1996). Some of such anomalies are
 391 observable in the cranial and ventral region (Loy et al.,
 392 2000; Tulli et al., 2009), fin erosion by erodibility
 393 (Arechavala-Lopez et al., unpublished data), otoliths

Table 6 Component loadings, percent of variance (% V) and eigenvalues for the principal components (with varimax rotation) in the Spain and Greece groups analyses for seabass

Spain	Greece							
	PC1	PC2	PC1	PC2	PC3	PC4	PC5	
F4	0.945		F3	0.904				
E4	0.905		Eye L	0.805				
D2	0.890		A3	0.801				
C2	0.887		A2	0.729				
C5	0.885		Eye H	0.725				
E3	0.884		B4	0.692				
C4	0.875		D5	0.543				
D4	0.872		B3	0.419				
E5	0.867		F2		0.724			
D5	0.859		F4		0.675			
C3	0.855		F1		0.671			
B2	0.851		C1		0.664			
E1	0.847		A1		-0.655			
E2	0.839		D3		0.583			
C1	0.835		C5		0.554			
B5	0.815		E2		0.491			
D1	0.812		D1			0.780		
B1	0.787		C2			0.748		
B3	0.771		D4			0.712		
D3	0.756		C4			0.627		
B4	0.697		B2			0.597		
F3		0.919	C3			0.581		
A3		0.914	E4				0.907	
A1		0.853	E1				0.885	
Eye L		0.830	E3				0.659	
A2		0.800	D2				0.626	
F2		0.695	E5				0.516	
Eye H		0.685	B1					
F1		0.632	B5				0.758	
% V	57.72	33.75		22.64	18.01	16.01	14.98	8.48
Eigenv.	16.74	9.79		6.56	5.22	4.64	4.34	2.46
ANOVA	<0.01	<0.01		<0.01	<0.01	0.24	0.11	0.03

Components with significant differences (ANOVA; $P \leq 0.05$) in morphological traits between farmed and wild seabass are marked in bold (see Table 2 for definition of characters)

394 and scale modifications (Carrillo et al., 2001; Arec-
 395 havala-Lopez et al., 2011c), and also bent body shape
 396 by skeletal anomalies, mainly in the haemal and
 397 caudal body regions (Paperna, 1978; Francescon et al.,
 398 1988; Balebona et al., 1993; Boglione et al., 1993,
 399 2001; Marino et al., 1993; Chatain, 1994; Koumoun-
 400 dourous et al., 1997; Loy et al., 1999, 2000; Afonso
 401 et al., 2000; Sfakianakis et al., 2006). However, wild-

402 caught seabream and seabass present a low number of
 403 malformations and are scarcely affected by any severe
 404 anomalies (Boglione et al., 2001; Loy et al., 2000). In
 405 this study, farmed fish were carefully selected from the
 406 captures looking for streamlined wild-like profiles,
 407 associated with absence or light anomalies cadres
 408 (Loy et al., 2000), to avoid these morphoanatomical
 409 differences cited above. Despite this, significant

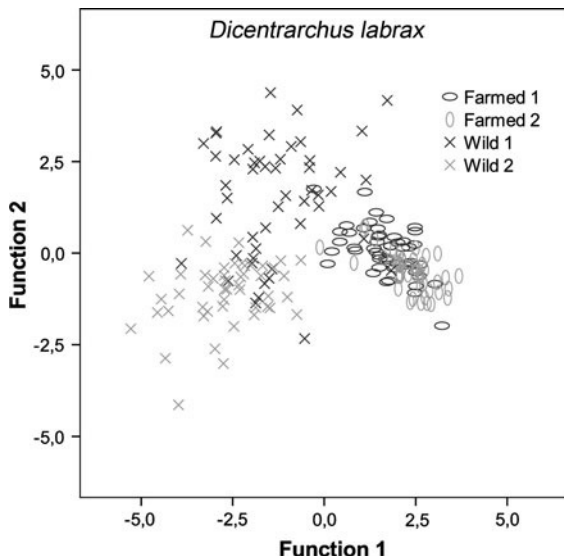


Fig. 7 Scatterplot of functions 1 and 2 for the discriminant analysis at Spain-group including the two locations of farmed seabass and the two localities of wild seabass

410 differences have been detected mainly in the anterior
411 region for both species, resulting from neither mal-
412 formations nor abnormalities.

413 In conclusion, this study provides evidences
414 that body morphology of seabream and seabass are

415 different according to their origin at different scales,
416 which could be attributed to both rearing environment
417 and genetic differentiation. Even more, the use of
418 morphoanatomical indices (K , CI and RP) seems to
419 have wider applicability than image processing (TNS)
420 in the identification of wild and farmed fish. Such
421 indices entail no great scientific expertise and they
422 have been shown to be a valuable tool for describing
423 changes in shape features, and could be used as a
424 useful technique in the field to identify escapees within
425 the wild stocks, and therefore, to monitor their
426 potential negative effects on the environment and
427 their influence on local fisheries landings. However, it
428 must be taken into account that an escapee that
429 survives over time may resemble a wild individual due
430 to the changes on habitat and food. The use of the
431 combined approach, such as morphometry, genetic
432 and other biological indicators (e.g. growth pattern of
433 scales and otoliths, fatty acids and trace elements),
434 should be considered for the more precise quantification
435 of escapes within natural populations, fisheries landing or
436 for evaluation of re-stocking programs. This will not only
437 contribute greatly to biological and ecological clarifica-
438 tion of the species but will also help to the development of
439 a strategy for natural stocks conservation and improving
440 the aquaculture sustainability.

Table 7 Inter-group classification score result (in %) for the discriminant analysis of the four groups of seabass

<i>Dicentrarchus labrax</i>	Group	Spain		Greece		Total
		Farmed	Wild	Farmed	Wild	
Origin group (%)	Spain farmed	98	2	0	0	100
	Spain wild	7	88	5	0	100
	Greece farmed	0	0	99	1	100
	Greece wild	0	1	0	99	100

Table 8 Data from the intra- and inter-groups discrimination analyses

Group	F	Eigenvalue	Percent variance	Cumulative variance	Canonical correlation	Wilks' λ	χ^2	df	P
Spain	1	4.405	77.5	77.5	0.903	0.071	485.064	81	<0.001
Spain	2	0.930	16.4	93.9	0.694	0.384	175.457	52	<0.001
Spain	3	0.348	6.1	100.0	0.508	0.742	54.848	25	<0.001
Greece	1	7.596	100.0	100.0	0.940	0.116	394.768	29	<0.001
Spain–Greece	1	9.818	70.4	70.4	0.953	0.011	1709.278	87	<0.001
Spain–Greece	2	3.227	23.1	93.5	0.874	0.124	798.482	56	<0.001
Spain–Greece	3	0.908	6.5	100.0	0.690	0.524	247.106	27	<0.001

The groups of seabass were classified from these functions (F) in the discriminant analysis

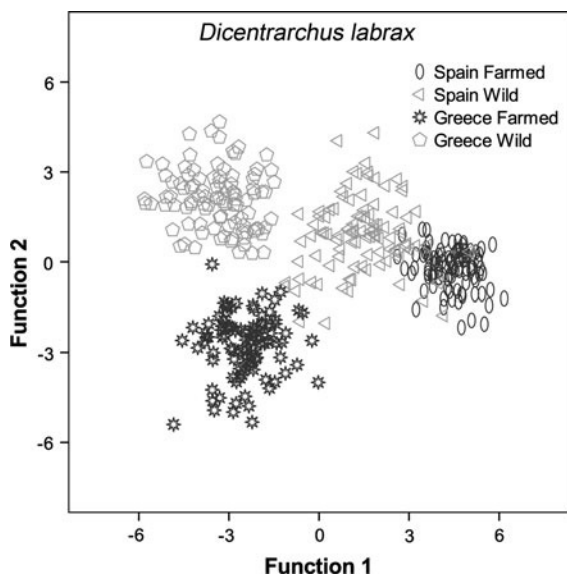


Fig. 8 Scatterplot of functions 1 and 2 for the discriminant analysis including the groups of farmed and wild seabass from Spain and Greece: *white circle* Spain Farmed; *white triangle* Spain Wild; *black star* Greece Farmed; *grey pentagon* Greece Wild

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