Geographic morphological variation within and between colour phases in *Coris julis* (L. 1758), a protogynous marine fish species

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Abstract

The possible differences between sexes in patterns of morphological variation in geographic space have been explored only in gonochorist freshwater species. We explored patterns of body shape variation in geographic space in a marine sequential hermaphrodite species, *Coris julis* (L. 1758), analyzing variation both within and between colour phases, through the use of geometric morphometrics and spatially-explicit statistical analyses. We also tested for the association of body shape with two environmental variables, temperature and chlorophyll a concentration, obtained from time-series of satellite-derived data. Both colour phases showed a significant morphological variation in geographic space and patterns of variation divergent between phases. In fact, while the morphological variation was qualitatively similar, individuals in the initial colour phase showed a more marked variation than individuals in the terminal phase. Body shape showed a weak but significant correlation with environmental variables which was more pronounced in primary specimens.

Keywords: geometric morphometrics – spatial analysis – body shape – geographic variation – ecomorphology – sexual dimorphism
Introduction

Phenotypic variation in geographic space can be the consequence of, in addition to other evolutionary factors such as gene flow or genetic drift, variation in environmental variables which, in turn, can influence phenotypic traits both directly and indirectly through trait correlations and interactions with other environmental factors (Lande & Arnold, 1983; DeWitt & Langerhans, 2003; Langerhans, Chapman & Dewitt, 2007). Moreover, the effect of environmental variables on phenotypes can lead to divergent patterns among populations which may or may not be parallel between sexes. That is, given that the majority of the genome is shared between males and females, sexes might show similar patterns of population divergence, but, conversely, differences between sexes (such as in morphology or behaviour) may interact with environmental gradients to produce divergent patterns of phenotypic variation between sexes in geographic space (Hendry et al., 2006).

Body shape variation in fish can reflect ecological and behavioural differences both within and between species, so shape is of particular interest in ecological and evolutionary research (Burns, Di Nardo & Hood, 2009). In particular, intraspecific variation in fish body shape has been related to various factors such as water flow (Langerhans, 2008), feeding habits (Ruehl & DeWitt, 2005), habitat use (Svanbäck & Eklöv, 2003, 2004), predation pressure (Gomes & Monteiro, 2008), temperature (Beacham, 1990; Loy et al., 1996) and diet (Wimberger, 1992). Morphometric analyses have been useful in highlighting patterns of stock differentiation in geographic space (Cadrin & Silva, 2005; Cadrin, 2010; Cadrin et al., 2010), even in cases where stock differentiation was undetectable by genetic studies (Levi et al., 2004; Salini et al., 2004). In particular, in the Mediterranean Sea, the morphometric analysis of body shape in Trachurus trachurus (L. 1758) has highlighted a structuring in three geographic groups (Murta, Pinto & Abaunza, 2008), a result which is in
agreement with analyses using other approaches (Abaunza et al., 2008). However, analyses of body shape variation at the Mediterranean scale are rare as most of studies on body shape focus on a smaller spatial scale.

One of the most studied factors affecting body shape in fish is temperature, which typically results in deeper bodies at higher temperatures and more elongated bodies at lower temperatures (Loy et al., 1996; Marcil, Swain & Hutchings, 2006). A recent study on Danio rerio (Hamilton, 1822) has also evidenced how rearing temperature during the larval phase can have consequences on adult body shape (Georgia & Koumoundouros, 2010). While many studies have focused on the plastic response of a single genotype to temperature (phenotypic plasticity), Kavanagh et al. (2010) have shown that temperature can induce in Thymallus thymallus (L. 1758) genetically-based phenotypic divergence even under conditions of low genetic variation and ongoing gene flow. Temperature can also be correlated with trophic resources: sampling sites with temporally persistent above-average chlorophyll a concentration and below average sea surface temperature have been considered productivity hotspots and have been found to be potentially associated with fisheries resources (Valavanis et al., 2004). Chlorophyll a variability is also associated with feeding intensity in sardine (Garrido et al., 2008). Despite the fact that time series of sea temperature and chlorophyll a data are now easy to obtain as satellite-derived data, such data have not been widely used to study patterns of morphological variation in the field.

While patterns of geographic variation in body shape have been studied in both marine and freshwater species, the possible differences between sexes in such patterns have been explored, to the best of authors’ knowledge, only in gonochorist freshwater species (Hendry et al., 2006; Langerhans & Makowicz, 2009). In fact, while various studies on the morphometric variation in hermaphrodite fish species exist (Vidalis, Markakis & Tsimenides, 1997; Moran, Burton & Caputi, 1999; Palma & Andrade, 2002, 2004), they do not use advanced techniques such as the combination of geometric morphometrics and...
spatial analysis and they do not test for differences between sexual phases in patterns of geographic variation.

The Labridae family is the third largest family of marine fish comprising species which occur worldwide in tropical, subtropical and temperate seas (Parenti & Randall, 2000). The phenomenon of sexual inversion, which is often associated to a change in colour, is very common in labrids (Warner & Robertson, 1978). *Coris julis* (L. 1758) is a small protogynous diandric labrid fish, which is common and almost continuously distributed in most of the Mediterranean Sea. The species can also be found in the eastern Atlantic European and North African coasts, where it can live in sympathy with its sister species *C. atlantica* (Gunther, 1862) (Parenti & Randall, 2000). *C. julis* is mainly found in rocky coastal areas and *Posidonia oceanica* (L.) Delile, 1813 beds and it is known to have a burrowing behaviour on sandy bottoms at night (Tortonese, 1970). The species is known to ingest gastropods, crustaceans, bivalves, small fish, polychaetes, echinoderms and benthic algae (Tortonese, 1970; Pinnegar & Polunin, 2000; Kabasakal, 2001) and is considered omnivorous with a preference for animal material (Karachle & Stergiou, 2008). Moreover, Vizzini & Mazzola (2009) have documented for the species a certain degree of variation in the trophic level index among geographically close sampling localities. Guidetti & D’Ambrosio (2004), studying the distribution patterns of *C. julis* and *Thalassoma pavo* (L. 1758), another labrid species with similar trophic traits, hypothesized that *T. pavo*, being more markedly thermophilic, outcompetes *C. julis* in shallower waters. *C. julis* juveniles have been repeatedly reported to act as facultative cleaners (Van Tassell, Brito & Bortone, 1994; Zander & Nieder, 1997; Zander & Sötte, 2002; Fischer et al., 2007), a behaviour that may be correlated to the presence of a lateral dark stripe (Arnal, Verneau & Dessevises, 2006). *C. julis* exhibits two colorations which are so different in morphology that they were originally described as separate species. The initial phase coloration (also called “primary...
livery”) is mainly exhibited by females and consists in a brown upper (dorsal) part of the body and a white ventral part; along with these main features there other sometimes less evident traits like a black and a yellow stripe along flanks. The terminal phase coloration (also known as “secondary livery”) is typical of males and is more colourful with a dorsal portion which is typically green (but also brown or bluish), a ventral white portion and an orange wavy band along flanks with a black elongated mark extending from about the pectoral fin to mid of the body. Initial and terminal colorations are, however, quite variable (Tortonese, 1970; Michel, Lejeune & Voss, 1987; Arigoni et al., 2002). Protogynous sex change is typically accompanied by colour change from the primary to the secondary coloration through a “transitional livery” phase with intermediate colour traits which is relatively short in duration (Bacci & Razzaudti, 1957; Bentivegna & Cirino, 1984). Behavioural observations (Lejeune, 1982; Bentivegna & Cirino, 1984) have shown that individuals with primary liveries are sedentary, individuals with secondary liveries are territorial and engage in agonistic behaviours when they meet each other, especially during the reproductive season. Social factors (male/female ratio) have been also shown to induce sexual inversion in the species which lacks of a critical size for sex inversion (Bentivegna & Cirino, 1984).

Despite the variation in coloration within livery and among sampling sites (Michel et al., 1987; Laurent & Lejeune, 1988; Arigoni et al., 2002) and the view of C. julis as a species that “presents conspicuous morphological variation between populations” (Aurelle et al., 2003), its intraspecific morphometric variation is still largely understudied. The variability in osteological trophic traits in two geographically close populations of the species has been recently assessed (Fruciano, Tigano & Ferrito, in press a) but, to the best of authors’ knowledge, the scientific literature is lacking of any other morphometric study on the intraspecific morphological variability of the species. Recent work on the species (Fruciano et al., in press b) has shown the absence of neutral genetic structuring within the
Mediterranean Sea which has been suggested to be the consequence of the high dispersal capabilities of the species, which are, in turn, probably correlated to the long planktonic larval phase (Gordoa, Moli & Raventos, 2000; Raventos & Macpherson, 2001).

The aims of the present study are to test if, despite the absence of neutral genetic structuring, *C. julis* morphology varies in geographic space at the Mediterranean scale and if primary and secondary individuals show different patterns of variation, and also to test for the association with environmental parameters. We expect a significant variation of shape in geographic space for both colour phases with a deeper body at sites with higher temperatures. We also expect that, as already observed in gonochorist freshwater species, patterns of variation in primary and secondary individuals are different because the two colour phases are subjected to different pressures.

To test these expectations, a geometric morphometric analysis of body shape variation among nine Mediterranean sampling sites has been performed using, in addition to other methods, spatially explicit analytical tools such as bearing analysis (Falsetti & Sokal, 1993) and trend surface analysis (Krumbein, 1959; Sneath, 1967; Gittins, 1968; Watson, 1971). While Mantel tests of the correlation between morphometric and geographic distances have been used widely in morphometric studies, the use of other spatially-explicit methods with geometric morphometric data is still in its infancy, despite of its advantages. In fact, bearing analysis, which detects the direction in geographic space of greatest correlation of morphometric and geographic distances, has never been used with morphometric data. Similarly, trend surface analysis, which allows to model the variation of one or more dependent variables in geographic space, has been used with geometric morphometric data only recently (Cardini, Jansson & Elton, 2007; Cardini & Elton, 2009; Cardini *et al.*, 2010).
Materials and methods

For the present study a total of 223 C. julis specimens from 9 different mediterranean populations (Fig. 1; Table 1) was used. Shortly after collection, fish were preserved in 95% ethanol and brought to the laboratory for the acquisition of morphometric data.

Data gathering and dataset preparation

Pictures of the left side of each specimen were taken using a digital camera mounted on a copy stand with an experimental design in which every specimen had two presentations (two pictures) and two digitizations of landmarks for each presentation, for a total of 4 sets of coordinates (such a design was deemed appropriate following the results of a preliminary study of measurement error on a subset of specimens). Throughout the data gathering phase, several measures have been taken to minimize, as much as possible, bias and error: the digital camera was distant from the specimens to reduce the effect of parallax (Mullin & Taylor, 2002), fish were kept straight by running a long needle of appropriate length through the right side of the body (Windsor Aguirre, personal communication), all the steps of the analysis have been performed by the same operator, populations were not photographed and digitized at a single session (e.g. all the specimens of a certain population in a single day) but in “rounds” of subsamples to avoid the effects of a potential bias in the way the operator performed his tasks (Windsor Aguirre, personal communication); further details on methodological steps are provided by Fruciano (2009).

Twenty points (both landmarks and semilandmarks; Fig. 2) were digitized using the software tpsDig (Rohlf, 2006). The landmark/semilandmark configurations were then subjected to a generalized Procrustes analysis with sliding semilandmarks (Bookstein, 1997) using the software tpsRelw (Rohlf, 2007a) setting ten iterations and the minimization of the squared
Procrustes distance as sliding criterion because this criterion removes all the tangential variation along outlines (Perez, Bernal & Gonzalez, 2006).

To reduce both directional and non-directional measurement error, the full dataset comprising four landmark configurations for each specimen has been first subjected to the procedure described in Valentin et al. (2008), then the resulting coordinates of each specimen (now adjusted for body arching) have been averaged so to obtain a single landmark configuration for each specimen. Average centroid size measures were also computed for each specimen. Given the marked difference in shape between primary and secondary individuals and following the results of a preliminary study, all subsequent analyses were performed separately for primary and secondary specimens. The program Standard6 of the IMP package (Sheets, 2002) was then used to obtain a dataset in which size-related allometric variation had been removed by regression on centroid size. The resulting configurations of points were then subjected to a principal component analysis (PCA) and PCA scores were used in subsequent analyses.

**Shape variation among populations**

To analyse body shape variation among populations two Multivariate Analyses of Variance (MANOVA; one for primary individuals and the other for secondary individuals) were performed using STATISTICA (StatSoft Inc) on size-adjusted PCA scores. Pairwise differences between populations were also tested using the permutation procedures on Mahalanobis distances implemented in the CVA module of MorphoJ (Klingenberg, 2011) testing the null hypothesis that the difference was zero. Only sampling sites with at least five specimens were subjected to MANOVA.
To test for differences between colour phases in the pattern of morphological variation between populations, a MANOVA testing for the interaction of livery and site was carried out with STATISTICA on the samples containing at least five specimens of each colour phase.

**Analysis of spatial patterns**

To analyse the patterns of shape variation in geographic space, three different spatially-explicit approaches were used: Mantel tests (Mantel, 1967), bearing analysis (Falsetti & Sokal, 1993) and trend surface analysis (Krumbein, 1959; Sneath, 1967; Gittins, 1968; Watson, 1971).

The Mantel test (Mantel, 1967) was used to test the null hypothesis that the correlation between pairwise geographic distances and pairwise tangent Procustes distances between populations was zero. Both geographic distances from geographic coordinates and shortest waterway distances among sampling locations were used. The analysis was carried out both using mean shapes for each livery and each sampling site and using all specimens of a certain colour phase. The test was performed with NTSYSpc 2.2 (Rohlf, 2007b) and PASSaGE 2 (Rosenberg & Anderson, 2011) using 10000 permutations.

Bearing analysis is a method of determining the direction of greatest correlation between data distance and geographic distance that has been introduced by Falsetti & Sokal (1993) for the use with genetic data. In bearing analysis, each geographic distance between two points is weighted by the squared cosine of the angle between the line connecting the two points and a reference vector. Then the correlation between the matrix of spatial distances weighted in this way and the matrix of data distances (morphometric distances, in this study) is computed and can also be tested using a Mantel test. The same calculations are then repeated changing the direction of the reference vector and the results can be plotted in a graph where the obtained correlation is depicted as function of the reference direction used for weighting. For the present study, bearing analysis has been performed with
PASSaGE 2 using pairwise tangent Procrustes distances among individuals as data
distances. To visualize morphological variation predicted by bearing analysis, the software
tpsRegr (Rohlf, 2007c) was used to regress shape variables on the projection of each
locality on the geographic direction of greatest correlation between morphometric and
geographic distances and to produce three-times exaggerated thin-plate splines of
predicted shapes at each extreme. Bearing analysis has been carried out only on
populations with at least five usable specimens.

Trend surface analysis is one of the most powerful tools of spatial pattern analysis
(Legendre & Legendre, 1998) but it has been introduced in the geometric morphometrics
field only recently by Cardini and colleagues (Cardini et al., 2007; Cardini & Elton, 2009;
Cardini et al., 2010). Trend surface analysis consists of the application of polynomial
regression to spatial data. In practice, it is a regression of one or more response variables
(in the case of the present study shape variables) on a polynomial expansion of
geographic coordinates (longitude and latitude, usually centred before the actual analysis).

Being of interest in the present study the broad scale trend of shape variation, a
polynomial of third degree was deemed appropriate following Legendre & Legendre (1998)
and the model $X+Y+X^2+XY+Y^2+X^3+Y^3$ (where $X$ represents the mean-centred longitude, $Y$ the
mean-centred latitude) was used as full model. Three different approaches to find a model
with less parameters were used: the Legendre & Legendre (1998) stepwise method, a
Using the Legendre & Legendre (1998) method, shape variables (principal component
scores) were regressed on all the terms of the full model, eliminating backwards terms not
significant at the 5% probability level one at a time. In case more than one variable was
non-significant in the regression the one to be removed was chosen based on the F-
statistic value (the variable with lowest F-statistic value was removed first). In the modified
version of the Legendre & Legendre (1998) method the only difference was that the
significance level to determine exclusion was 1% (p=0.01) so to reduce further the number
of variables in the chosen model. Finally, AICc (Hurvich & Tsai, 1989) is a modified version
of the AIC (An Information Criterion; Akaike, 1973), adjusted for small sample sizes. The
script provided by Al-Subaihi (2002) was used to select the best model using the AICc
criterion. All the regressions were performed using the software STATISTICA. For
visualization purposes, predicted values for relative warp scores were computed and back-
transformed to xy-coordinates, then a five-times exaggerated thin-plate spline of each
predicted shape on the consensus shape was produced.

Tangent Procustes distances between all pairs of predicted shapes were then computed
with tpsSmall (Rohlf, 2003) and the correlation between the matrices of distances between
predictions in primary and secondary specimens was then tested by the means of a
Mantel test.

**Association of body shape with ecological variables**

Time-series of chlorophyll a concentration (October, 16th, 1987-October, 16th 2007;
monthly interval) and sea surface temperature (June, 7th, 2004-January, 5th, 2009; weekly
interval) at the sampling locations were obtained from satellite data of the American
National Aeronautics and Space Administration (NASA). Given that the chlorophyll
concentration data were incomplete, only the sampling sites for which more than 40% of
the time series was available were used in analyses using this variable, so discarding
specimens from Oristano, Pantelleria and Riposto. Arithmetic mean sea surface
temperature and geometric mean chlorophyll a concentration (Supplemental material S1)
were used as variables choosing geometric mean for chlorophyll a concentration because
chlorophyll a distributions tend to be roughly log-normal and it has been noticed that using
geometric mean is a better estimator of central tendency (MacFadyen, 1998).
The association of shape with chlorophyll a concentration and sea surface temperature was studied with two-block partial least squares analysis using both ecological variables and also with separate regressions of shape on each ecological variable. Separate regressions of shape variables on chlorophyll a concentration and sea surface temperature were performed using tpsRegr visualizing statistically significant regressions with thin-plate splines of three-times the maximum and minimum observed values relative to reference.

A two-block partial least squares analysis using as first block the shape variables and as second block the two ecological variables (standardized to unit variance) was performed with MorphoJ and the significance of the association of morphometric and ecological variables was obtained by the permutational procedure implemented in the program (10000 permutations). Shape variation predicted by partial least squares analysis was visualized with MorphoJ producing “wireframe graphs”.

Results

Shape variation among populations

MANOVA rejected the null hypothesis of no difference between population means both in primary (Wilks’ Lambda= F234,329.47=2.029 p<0.001) and secondary (Wilks’ Lambda=0.09 F273,482.11=1.752 p<0.001) specimens. The pairwise comparison among sampling sites using primary specimens is always significant (p<0.001). Pairwise comparisons using secondary specimens is always significant (p<0.001) except in the case of the comparison between Augusta and Lecce samples.

The MANOVA testing for the interaction effect of colour phase and sampling site is also highly significant (Wilks’ Lambda=0.0001 F320,536.283=3.551 p<0.001).
Analysis of spatial patterns

Correlations between shape distances and geographic distances (both as linear distances from coordinates and shortest waterway distances) using average shapes for each sampling site are very low (correlation coefficients ranging from 0.05 to -0.14) and Mantel tests shows that in no case such correlations are statistically significant. On the other hand, correlations are significant when using all specimens separately (that is, the observed Z value is higher than most of the Z values obtained from the permutational procedure; p-value comprised between 0.01 and 0.0004). Also, except in the case of primary specimens and shortest waterway distances (where the correlation is 0.09), the other correlations between morphometric and physical distances using separate specimens are, albeit still low, much higher (range 0.11-0.14) than the ones observed using sampling site means.

The bearing analysis of primary individuals showed that the direction of highest significant correlation between geographic and morphometric distances is 30° (correlation 0.1206, permutational p-value 0.002), corresponding to a direction NE-SW. The bearing analysis of secondary individuals suggests that the direction of highest significant correlation is 111° (correlation 0.1535, permutational p-value <0.001), corresponding to a direction NW-SE. However, the plots of correlations obtained using the various reference directions (Supplemental material S2) show that there are significant correlations also along different directions in geographic space.

The regression of shape variables on projections of sampling localities on the direction of highest correlation explains only 3.74% of variance in primary individuals, 5.14% in secondary individuals. Body shape variation along the directions of highest correlation is depicted in Fig. 3.

The polynomial expansion of latitude and longitude terms performed, as expected, better than a simple regression on latitude and longitude (which would have explained for
primary and secondary specimens respectively 9.73% and 8.11% of variance). Table 2 shows the results of trend surface analysis while Fig. 4 and 5 show shapes predicted from TSA at sampling sites using the model selected by AICc (the other models, while accounting for slightly more variance, produced similar patterns and will not be shown). The mean tangent Procrustes distance between shapes predicted by AICc is 0.009 for primary specimens, 0.006 for secondary individuals. The correlation between tangent Procrustes distances among predictions of primary and secondary specimens is high and significant (r=0.77, p=0.011).

**Association of body shape with ecological variables**

Results of the partial least squares analysis are provided in Table 3 and show a weak but significant association between shape and environmental variables. Both in primary and secondary specimens temperature has a positive correlation while chlorophyll has a negative correlation with the first pair of singular axes. Shape changes predicted by the association of shape and environmental variables are depicted in Fig. 6. Separate linear regression of primary specimens' body shape on sea surface temperature and chlorophyll a concentration are both significant (respectively p=0.0028 and p=0.002) and explain respectively 5.33% and 5.6% of variance; Fig. 7 depicts shape changes predicted by these two regressions. Linear regression of secondary specimens' body shape on chlorophyll a concentration was significant using parametric tests (p=0.037) but non significant using permutational approaches (0.06) so, given also the extremely low explained variance (1.6%), the regression was deemed non-significant. Regression on sea surface temperature, on the other hand, is highly significant (p=0.002) and accounts for 3.9% of variance; Fig. 8 shows shape changes predicted by the regression of body shape on sea surface temperature in secondary individuals.
Discussion

Looking at the results of the MANOVA analyses, it can be said that overall the populations are differentiated from each other.

While the results of Mantel tests should be interpreted with caution as this test has been shown to be affected by both sample size and inequality of sample sizes (Luo & Fox, 1996), the fact that correlations between geographic and morphometric distances are small suggests that in *C. julis* shape differences in geographic space cannot be explained as mere consequence of the distance among sites. The results of bearing analysis show that, along certain directions in geographic space, there is a significant correlation between geographic and morphometric distances, thus suggesting the existence of clines of shape variation. Interestingly, the directions of maximum correlations are different in primary and secondary individuals. The results of trend surface analysis suggest a complicated pattern of variation in geographic space because the polynomial expansion of latitude and longitude terms provides a much better fit to the data (so a simple model describing a linear effect along a direction in geographic space is not completely adequate). The fact that a simple directional gradient in geographic space is not adequate to model shape variation in *C. julis* is also corroborated by the higher levels of explained variance in trend surface analysis compared to variance explained by regression of shape on the projection of sampling sites on the directions of maximum correlation resulting from bearing analyses. While a simple linear effect is not adequate to model shape variation in geographic space, looking at the shapes predicted by trend surface analysis at selected sites for primary specimens, it is possible to discern a pattern. In fact, it can be noticed that the Split population has the most elongated and narrow predicted shape and Naples and Lecce have also an elongated and narrow predicted shape (but not to the extent of the Split sample); all the more southern samples show in general a deeper body. Similarly,
Oriostano shows, compared to the Mallorca population, a more elongated shape (still not to the extent of the Split sample). Trying to capture an easily explainable general trend, it could be said that at similar longitudes northern populations show a narrower and more elongated body shape, at similar latitudes eastern populations show a narrower body. It is important to remember, however, that such a “general overview” represents an oversimplification of a more complex, statistically significant, pattern at the studied geographic scale. In secondary individuals, it can be said that the pattern is somewhat similar, but less marked, to the one found in primary individuals with Split having narrower bodies than the other samples. While the inclusion of more sampling sites would probably have allowed for a more precise modelling of morphometric variation in geographic space, it is remarkable that all the methods used agree in detecting a variation in geographic space.

The analysis of the association between body shape and environmental variables show a weak but significant correlation. In primary individuals, given the different sign of the coefficient on the first singular axis for chlorophyll a concentration and sea surface temperature, the thin-plate spline visualizing shape changes along the first singular axis suggests that, in areas with higher chlorophyll a concentration and lower temperature, individuals have a narrower, more elongated body. Predicted shapes for the separate regressions on chlorophyll concentration and temperature reveal a similar pattern.

Considering that high chlorophyll a concentration and low sea surface temperature are associated to productivity hotspots (Valavanis et al., 2004) and that chlorophyll has been shown to be associated to phytoplankton fraction and feeding intensity in sardine (Garrido et al., 2008), it is possible that body shape of primary individuals of *C. julis* might be influenced by productivity and/or correlated variables.

In secondary individuals, while the association between shape and ecological variables is significant, the correlation between temperature and the first singular axis is extremely
high while the negative correlation of chlorophyll a concentration with the first singular axis is low. Moreover, considering separate regressions, only the regression on sea surface temperature is significant and the predicted shapes show that individuals inhabiting environments with lower sea surface temperature exhibit a more elongated shape while individuals from habitats with higher sea surface temperature exhibit a deeper body, the same general pattern shown by primary individuals in the regression on sea surface temperature. The variation in body shape observed in both primary and secondary individuals might have functional significance. In fact, a more elongated shape minimizes drag and is predicted to be favoured in case of steady swimming, while a deeper body guarantees higher efficiency in case of unsteady swimming with rapid bouts of activity (Walker, 1997; Langerhans, 2008). Variation in *C. julis* body shape might also be affected by the burrowing behaviour which this species exhibits but such an effect should be tested thoroughly. A more elongated shape in environments with lower sea surface temperature might be caused by a variation in the number of vertebrae, which has been shown to increase with latitude in many fish species (Jordan, 1891; McDowall, 2008) and which can have both genetic and phenotypically plastic components (Yamahira, Lankford & Conover, 2006; Yamahira & Nishida, 2009). Temperature has been found to have an effect on body shape in various fish species (Beacham, 1990; Loy *et al*., 1996; Marcil *et al*., 2006; Georgia & Koumoundouros, 2010; Kavanagh *et al*., 2010). Beacham (1990) in a study on *Oncorhynchus keta* (Walbaum, 1792) found that temperature had a marked effect on juvenile morphometric variation and higher rearing temperatures have been found to determine deeper bodies in *Cyprinus carpio* L. 1758 juveniles (Loy *et al*., 1996). Similarly, Marcil *et al.* (2006) found in *Gadus morhua* L. 1758 that temperature and food abundance had an effect on body shape: individuals reared at higher temperature or food level had a deeper body and a larger head than those reared at the lower temperature or food supply, with the effect of temperature being more pronounced. Marcil *et al.* (2006), noticing that an
increase in body depth has been found to be associated with higher temperature in various fish species, have also suggested that such a response might be general in fish. Wimberger (1992) found that different diets produced different morphologies in two species of the genus Geophagus (Teleostei, Cichlidae). In the light of the above-mentioned previous studies on the effect of diet and temperature on body shape, it is possible to hypothesize that in C. julis temperature and chlorophyll a concentration have direct and/or indirect effects. In fact, while a direct effect of temperature is easily hypothesizable given that this environmental factor is known to act directly on body shape (Beacham, 1990; Marcil et al., 2006; Georgia & Koumoundouros, 2010), it seems more reasonable that the chlorophyll a concentration is associated with some other, presently unknown, variable which causes the variation in shape. The significant effect of chlorophyll a concentration obtained in the present study might also be a mere consequence of its negative correlation with temperature. Moreover, the fact that the association between body shape and the environmental variables studied is weak suggests that there may be other factors affecting the geographic variation of body shape in C. julis. Another possible factor affecting body shape variation in geographic space which should be investigated in future studies is the variation of biological communities in geographic space. In particular, Thalassoma pavo, being more markedly thermophilic, is believed to outcompete C. julis in shallow waters (Guidetti & D’Ambrosio, 2004) and the abundance of T. pavo has been shown to decrease at northern sites (Guidetti et al., 2002). It is, therefore, possible that the extent of the competition between C. julis and T. pavo varies with temperature in geographic space and that this competition drives, at least in part, the morphological variation observed in C. julis. The present study evidences also interesting differences between primary and secondary individuals both in patterns of variation in geographic space and in the association with environmental variables. This is highlighted by the significant interaction between colour
phase and sampling site in MANOVA. Moreover, while the similarity of patterns of
geographic variation in primary and secondary individuals is evident from the examination
of plots of predicted shapes and the high correlation of morphometric distances between
predicted shapes in primary and secondary individuals, the patterns of variation are less
marked in secondary specimens (as shown by the smaller average distance between
predictions). Moreover, all the chosen models in trend surface analysis account for more
variation in primary specimens. This discrepancy might be explained hypothesizing that
the secondary specimens are less prone to factors that shape variability at the studied
geographical scale.

The fact that in primary specimens both separate regressions on ecological variables are
significant while in secondary specimens only the regression on sea surface temperature
is statistically significant suggests the hypotheses that secondary individuals are either
less sensitive to the studied ecological variables or that they are influenced by these
environmental variables in a more complex way, not revealed by the analyses performed.

It is possible to hypothesize that the difference between primary and secondary individuals
in patterns of variation in geographic space and association with ecological variables is a
consequence of a difference between liveries in constraints that determine morphological
variation. In fact, while primary specimens are gregarious and, probably, mostly interested
in obtaining food resources, secondary specimens are known to establish territories and
engage in agonistic behaviours with each other (Lejeune, 1982; Bentivegna & Cirino,
1984) so body shape in secondary individuals might also be influenced by other factors
and be the results of more complex trade-offs.

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References


This is the accepted version of the manuscript. The definitive version is available at http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8312.2011.01700.x/full.


584 **Kabasakal H. 2001.** Description of the feeding morphology and the food habits of four sympatric labrids (Perciformes Labridae) from south-eastern Aegean Sea, Turkey. *Netherlands Journal of Zoology* **51**: 439-455.


Figure legends

**Figure 1.** Geographic locations for the samples used in the study. AU=Augusta, SR, Italy; LE=Porto Cesareo, LE, Italy; ML=Badia de Palma, Mallorca, Spain; MA=Mazara del Vallo, TP, Italy; NA=Capo Posillipo, Naples, Italy; OR=Oristano, Italy; PN=Pantelleria, Italy; RI=Riposto, CT, Italy; SP=Split, Croatia

**Figure 2.** Landmark (filled circles) and semilandmark positions.

**Figure 3.** Three-times exaggerated thin-plate splines obtained to visualise results of the bearing analysis in primary (a, solid arrow) and secondary (b, dashed arrow). The splines show the predicted shape at three times each extreme versus the average shape for each livery (used as reference)

**Figure 4.** Predicted shapes for the trend surface analysis of primary specimens at the sampled locations.

**Figure 5.** Predicted shapes for the trend surface analysis of secondary specimens at the sampled locations.

**Figure 6.** Shape change predicted by partial least squares analysis. The figure shows shape change associated to an increase in temperature and a decrease in chlorophyll a concentration. The reference (average shape) is shown in light grey dashed line and empty circles.
Figure 7. Three-times exaggerated splines showing shape changes predicted by separate regressions of shape on chlorophyll a and temperature using primary specimens. a=negative chlorophyll extreme, b=positive chlorophyll extreme, c=negative temperature extreme, d=positive temperature extreme; in all splines the average shape of primary individuals has been used as reference.

Figure 8. Three-times exaggerated splines showing shape changes predicted by the regression of shape on sea surface temperature using secondary specimens. a=negative extreme, b=positive extreme.

S2. Bearing analysis: plot of correlations at different directions. Circles indicate significant correlations. While the bearing analysis to find the direction of highest correlation on which the splines are based has been carried out at 1 degree intervals for good resolution, the plot shows correlations only at 5 degree intervals to produce an easier to understand picture.
Tables

Table 1 Sampling data for each population used in the study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sampling time</th>
<th>Environment</th>
<th>Coordinates</th>
<th>Livery</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Split (SP)</td>
<td>March 2007</td>
<td>Posidonia oceanica beds; a few meters of depth</td>
<td>43°28'N 16°24'E</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Naples (NA)</td>
<td>February 2007</td>
<td>Gravel bottom; 10-12 meters of depth</td>
<td>40°46'N 14°12'E</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Lecce (LE)</td>
<td>May 2007 and May 2009</td>
<td>Patches of rocky bottom and Posidonia oceanica beds; 8-20 meters of depth</td>
<td>40°14'N 17°52'E</td>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>Oristano (OR)</td>
<td>April 2007</td>
<td>Rocky bottom with photophilic algae; Posidonia oceanica beds; 5-6 meters of depth</td>
<td>39°48'N 8°31'E</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>Mallorca (ML)</td>
<td>June 2007</td>
<td>Rocky bottom with photophilic algae; Posidonia oceanica beds; 9-17 meters of depth</td>
<td>39°31'N 2°39'E</td>
<td>21</td>
<td>40</td>
</tr>
<tr>
<td>Riposto (RI)</td>
<td>January 2007; March 2007; April 2008</td>
<td>Patches of rocky and sandy bottom; 4-8 meters of depth</td>
<td>37°43'N 15°13'E</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>Mazara del Vallo (MA)</td>
<td>June 2007</td>
<td>Posidonia oceanica beds; 3-5 meters of depth</td>
<td>37°38'N 12°35'E</td>
<td>14</td>
<td>19</td>
</tr>
<tr>
<td>Augusta (AU)</td>
<td>May 2008 and June 2008</td>
<td>Rocky bottom with few photophilic algae; 4-10 meters of depth</td>
<td>37°11'N 15°14'E</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>Pantelleria (PN)</td>
<td>October 2008</td>
<td>Rocks with Posidonia oceanica patches close to a sandy area with Posidonia; 5-15 meters of depth</td>
<td>36°50'N 11°59'E</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td>106</td>
<td>117</td>
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</table>
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Table 2 Results of the trend surface analysis on primary and secondary specimens; X=mean-centered latitudinal term; Y=mean-centered longitudinal term

<table>
<thead>
<tr>
<th>Method</th>
<th>Chosen model</th>
<th>Explained Variance</th>
</tr>
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<tbody>
<tr>
<td><strong>Primary specimens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full model</td>
<td>X+Y+X²+XY+Y²+X³+Y³</td>
<td>22.61%</td>
</tr>
<tr>
<td>Legendre &amp; Legendre (1998)</td>
<td>X+Y+X²+X³+Y³</td>
<td>20.33%</td>
</tr>
<tr>
<td>Modified Legendre &amp; Legendre (1998)</td>
<td>X+X²+X³+Y³</td>
<td>15.82%</td>
</tr>
<tr>
<td>AICc</td>
<td>X+Y³</td>
<td>12.86%</td>
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<tr>
<td><strong>Secondary specimens</strong></td>
<td></td>
<td></td>
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<tr>
<td>Full model</td>
<td>X+Y+X²+XY+Y²+X³+Y³</td>
<td>17.79%</td>
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<tr>
<td>Legendre &amp; Legendre (1998)</td>
<td>X+Y+X²+Y²+X³+Y³</td>
<td>15.4%</td>
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<tr>
<td>Modified Legendre &amp; Legendre (1998)</td>
<td>X²+Y²+Y³</td>
<td>11.21%</td>
</tr>
<tr>
<td>AICc</td>
<td>X²+Y²</td>
<td>9.39%</td>
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Table 3 Results of the partial least squares (PLS) analysis in primary and secondary specimens. The RV coefficient (Escoufier, 1973) is a multivariate analogue of the squared correlation.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Correlation between blocks</th>
<th>Results for each PLS singular axis</th>
<th>Correlation of environmental variables with each PLS axis</th>
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<tr>
<td></td>
<td>RV</td>
<td>p-value</td>
<td>Axis</td>
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<tr>
<td>Primary specimens</td>
<td>0.1877</td>
<td>0.0008</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
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<tr>
<td>Secondary specimens</td>
<td>0.1368</td>
<td>&lt;0.0001</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td></td>
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Primary specimens

Secondary specimens

Figure 6
Chlorophyll

Sea surface temperature
Sea surface temperature
Supplemental material S1: Satellite-derived ecological data. Sea surface temperature (SST) in Celsius degrees, chlorophyll a concentration in mg/m$^3$.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Average SST</th>
<th>Chlorophyll geometric mean</th>
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</thead>
<tbody>
<tr>
<td>Augusta</td>
<td>20.1825</td>
<td>0.359396216</td>
</tr>
<tr>
<td>Lecce</td>
<td>19.38583333</td>
<td>0.379711734</td>
</tr>
<tr>
<td>Mazara</td>
<td>19.33291667</td>
<td>0.393831368</td>
</tr>
<tr>
<td>Mallorca</td>
<td>19.9975</td>
<td>0.32646652</td>
</tr>
<tr>
<td>Naples</td>
<td>19.95833333</td>
<td>0.791435495</td>
</tr>
<tr>
<td>Oristano</td>
<td>18.98416667</td>
<td>-</td>
</tr>
<tr>
<td>Pantelleria</td>
<td>20.06625</td>
<td>-</td>
</tr>
<tr>
<td>Riposto</td>
<td>20.13041667</td>
<td>-</td>
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<tr>
<td>Split</td>
<td>18.41666667</td>
<td>0.501116893</td>
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