Body shape variation and colour change 1 during growth in a protogynous fish 2 3 Carmelo Fruciano, Concetta Tigano, Venera Ferrito 4 Dipartimento di Scienze Biologiche, Geologiche e Ambientali– University of 5 Catania, via Androne 81, 95124 Catania, Italy 6 7 **Corresponding author** 8 Carmelo Fruciano 9 Telephone +39 095 7306023 10 Fax +39 095 327990 11 e-mail c.fruciano@unict.it 12 current address: Laboratory for Zoology and Evolutionary Biology, Department of Biology, 13 University of Konstanz, Universitätsstrasse 10, 78464 Konstanz, Germany 14 15 **Keywords:** *Geometric morphometrics, Labridae, labrids, colour change,* 16 protogyny, parallel coordinates 17 18 Abstract Protogynous sequential hermaphroditism is very common in marine 19 fish. Despite a large number of studies on various aspects of sequential 20 hermaphroditism in fish, the relationship between body shape and colour during 21 growth in dichromatic species has not been assessed. Using geometric 22 morphometrics, the present study explores the relationship between growth, body 23 shape and colouration in Coris julis (L. 1758), a small protogynous labrid species 24 with distinct colour phases. Results show that body shape change during growth is 25 independent of change in colour phase, a result which can be explained by the 26 biology of the species and by the social control of sex change. Also, during 27 growth the body grows deeper and the head has a steeper profile. It is 28 hypothesized that a deeper body and a steeper profile might have a function in 29 agonistic interactions between terminal phase males and that the marked 30 chromatic difference between colour phases allows the lack of strict 31 interdependence of body shape and colour during growth.

## 32 Introduction

33 Hermaphroditism is widespread in marine fish where it is present both as simultaneous and 34 sequential hermaphroditism (Shapiro 1979; Warner 1984). While many aspects of sequential 35 hermaphroditism, e.g. gonadal tissutal and morphological variation, body colour variation and size 36 at sex change, in fish have been extensively studied, to the best of the authors' knowledge the 37 relationship between body shape and colour has not been assessed in fish species in which 38 different sexual phases show different colouration. This has happened despite of the fact that the 39 size-advantage hypothesis of sex change and its variations have been extensively explored 40 (Warner 1984,1988; Muñoz and Warner 2003,2004; Warner and Muñoz 2008) and despite of the 41 fact that both body shape feature and colouration are correlated to reproductive or territorial 42 success in a few fish species (Warner and Schultz 1992; Kuwamura et al. 2000). The family 43 Labridae comprises species in which the sexes differ in colour. The first colouration, usually 44 associated to females, is often called "primary livery" while the second colouration, usually 45 associated with males, is called "secondary livery" (Tortonese 1970). Various labrid species are 46 also diandric - that is, there are individuals, called "primary males", which are males but present 47 the primary livery (Reinboth 1967; Warner and Robertson 1978). Coris julis (Linnaeus 1758) is a 48 small protogynous diandric labrid species, which is common along most of the Mediterranean Sea 49 coasts but also inhabits Eastern Atlantic coasts. Recent studies have shown that C. julis lacks 50 genetic structuring at the Mediterranean scale (Fruciano et al. 2011a) but nonetheless shows 51 regional morphometric variation (Fruciano et al. 2011b; Fruciano et al. *in press*) and that patterns 52 of morphological variation in geographic space can be different between colour phases. Coris julis 53 also shows a certain degree of variability in colour pattern. In fact, primary individuals are known 54 to vary in colour from a brown-based pattern to a reddish pattern as water depth increases and have 55 been found to be greener in Caulerpa taxifolia meadows (Michel et al. 1987; Arigoni et al. 2002). 56 The primary and secondary liveries of C. julis are so different that they were originally described 57 as two different species: Labrus giofredi (Risso, 1810) and C. julis (L. 1758). Behavioural 58 observations (Lejeune 1982; Bentivegna and Cirino 1984) have shown that individuals with 59 primary liveries are sedentary, while individuals with secondary liveries are territorial and engage 60 in agonistic behaviours when they meet each other, especially during the reproductive season. 61 Social factors (male/female ratio) have been shown to induce sexual inversion in the species 62 (Bentivegna and Cirino 1984). Histological aspects of sex inversion in C. julis have been described

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63	by Bruslé (1987) and Bentivegna et al. (1985), who also noticed a correlation between colour
64	phase and gonadic state. The variation in colouration during sex change, which creates a
65	transitional form with intermediate colouration traits called "transition livery", has been described
66	by Bentivegna and Cirino (1984) who also commented that the changes in colour marks during
67	transition do not always follow the same order. It is unclear, however, if the hormonal changes
68	which are believed to result in colour (and sex) change in this and other labrid species (Reinboth
69	1975,1988; Reinboth and Brusle-Sicard 1997; Frisch 2004; Ohta et al. 2008) at the same time also
70	cause a variation in body morphology or if the two processes are at least partially independent. In
71	fact, given that certain features of body shape have been shown to be important in territorial
72	interactions among secondary males (Warner and Schultz 1992), it could be expected that a change
73	in sex and colouration would result at the same time in a change in body shape. For this reason,
74	analysing the relationships between size, shape and colouration – as opposed to studying only one
75	trait at a time – can help understanding the relative importance of each morphological feature in
76	the biology of hermaphrodite fish species. Therefore, the aim of this study was to determine if
77	colouration and body shape changes happen simultaneously by using geometric morphometrics
78	coupled with both exploratory and hypothesis-testing statistical tools.
79	

### 80 Materials and methods

### 81 Dataset preparation

82 For the present study, a total of 263 *Coris julis* specimens, sampled with fish traps, nets, fishing

83 rods and hand lines at 9 different Mediterranean sites (Fig. 1; Table 1), was used. Fish were

84 preserved in 95% ethanol and brought to the laboratory for the acquisition of morphometrical data.

85 The colour phase of each specimen was determined by visual inspection of the colour marks,

86 identifying as transitional the individuals with colour patterns intermediate between primary and

87 secondary individuals, as described in Bentivegna and Cirino (1984).

88 Pictures of the left side of each specimen were taken using an Olympus C-3030 digital camera

89 mounted on a copy stand. Each specimen was photographed two times and points were digitized

90 two times for each pictures, obtaining a total of 4 sets of coordinates per specimen (such a design

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91	was deemed appropriate following the results of a preliminary study on measurement error on a
92	subset of specimens). In the course of the data gathering phase, several measures have been taken
93	to minimize bias and error: the digital camera was relatively distant (495 mm) from the specimens
94	to reduce the effect of parallax (Mullin and Taylor 2002), fish were kept straight by running a long
95	needle of appropriate length through the right side of the body (Windsor Aguirre, pers comm) as to
96	limit dorso-ventral arching, all the steps of the analysis were performed by the same operator,
97	individuals of one population were not all photographed and digitized within the same session but
98	in different ones to avoid any bias in the way the operator performed his tasks (Windsor Aguirre,
99	pers comm); further details on methodological steps are provided by Fruciano (2009).
100	Twenty points (Fig. 2), comprising both landmarks (i.e. homologous points) and semilandmarks
101	(i.e. points which are not homologous but retain positional correspondence), were digitized using
102	the software tpsDig (Rohlf 2006). The landmark/semilandmark configurations were then subjected
103	to a generalized Procustes analysis with sliding semilandmarks (Bookstein 1997) using the
104	software tpsRelw (Rohlf 2007a), setting ten iterations and the minimization of the squared
105	Procrustes distance as sliding criterion because this criterion removes all the tangential variation
106	along outlines (Perez et al. 2006).
107	To reduce both directional and non-directional measurement error, the full dataset comprising four
108	landmark configurations for each specimen was subjected to the procedure described in Valentin et
109	al. (2008), then the resulting coordinates of each specimen (now adjusted for body arching) were
110	averaged in order to obtain a single landmark configuration for each specimen. Centroid size (the
111	square root of the summed squared distances of each landmark from the center of the form;
112	Bookstein 1989) was also computed for each of the four landmark configuration per specimen and
113	then averaged to obtain an average centroid size per specimen.

### 114 Statistical analyses

115 Body shape variation during growth was studied with two approaches: regression on a size

116 measure (centroid size) and exploratory plots of both relative warps and relative warps in size-

117 shape space (Mitteroecker et al. 2004).

118 Regression of shape variables on centroid size were performed with tpsRegr (Rohlf 2007b),

119 visualizing shape variation with a "wireframe graph" produced by the software MorphoJ

120 (Klingenberg 2011).

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Relative warps in size-shape space were computed as explained by Mitteroecker et al. (2004),

121	Kelative walps in size-shape space were computed as explained by initeroceker et al. (2004),
122	adding to the usual shape variables the natural logarithm of centroid size and then performing a
123	principal component analysis (PCA). Plots of individual scores on the first three PC axes were
124	finally obtained with the software STATISTICA (StatSoft, Inc.).
125	To further explore the relationships among size, shape and colour phase in more than three
126	dimensions of multivariate space, we also obtained parallel coordinate plots of relative warps.
127	Using parallel coordinates (Inselberg 1985; Wegman 1990) is an approach that allows a
128	visualization of data with more than three dimensions by avoiding the use of orthogonal axes and
129	substituting them with parallel axes. For each observation (individual) the value of a certain
130	variable is represented by a point on the corresponding vertical axis (each axis represents a
131	variable). The points for each observation on each axis are then usually linked by segments so that
132	each observation (individual) in a sample is represented by a poly-line with vertices on the axes
133	with the position of the i-th vertice corresponding to the value of the i-th variable. While the
134	parallel coordinates technique has been employed in various fields, especially for a visual
135	exploration of data, its use in biology has been rare (Shapley 2004) and, to the best of the authors'
136	knowledge, it has never been used with geometric morphometric data.
137	To check for possible bias in the analysis due to unequal sample sizes or to geographic variation,
138	we also carried out the above-mentioned analyses on a single population (Mallorca) and on a
139	subset of geographically close sampling sites (Augusta, Riposto, Mazara del Vallo, Pantelleria).
140	To quantify the degree of overlap between the portions of morphospace occupied by primary and
141	secondary individuals, we computed the ratio of the convex hull volume (the volume of the n-
142	dimensional minimal convex set enclosing a certain set of observation; see Cornwell et al 2006 for
143	an example in ecology) shared by both primary and secondary individuals on the total convex hull
144	volume for primary and secondary primary and secondary individuals. Convex hull computations
145	were performed on the first ten relative warps using the Quickhull algorithm (Barber et al 1996)
146	implemented in Octave (http://www.octave.org/).
147	

147

# 148 **Results**

149	The regression of shape on centroid size was highly significant (Wilk's Lambda 0.27; p<0.001)
150	and explained 23.5% of total variance. Fig 3 depicts body shape changes associated with changes
151	in size and shows a deepening of body during growth and that the relative positions of the eye and
152	the forehead change during growth with the eye closer to the forehead in larger specimens which
153	also show a steeper head profile.
154	In size-shape space, the first relative warp (principal component) was, as expected, highly
155	correlated with the logarithm of centroid size ( $r=0.99$ ). The scatterplot of relative warps in size-
156	shape space (Fig 4) shows that primary and secondary specimens tend to occupy different portions
157	of the multivariate space. Therefore, given that the first principal component has a very high
158	correlation with the size measure and given that successive axes are orthogonal to the first in
159	multivariate space, it can be argued that, if in general terms primary and secondary specimens
160	occupy different portions of the size-shape space, they are different both in size and in shape.
161	However, there is also a certain degree of overlap between the groups of primary and secondary
162	specimens. It is also evident that transitional specimens do not occupy a definite and exclusive
163	portion of the multivariate space - they are scattered across areas where either primary or
164	secondary specimens are prevalent.
165	A similar situation is evident observing the parallel coordinates plot of relative warp scores
166	(Supplementary Fig. S1), which allows visualization of more than three dimensions and where
167	there is a substantial overlap of portions of morphospace occupied by primary and secondary
168	specimens (with a few primary individuals in portions of the space where secondary individuals
169	are prevalent and vice-versa). Observing the parallel coordinate plot, it is also clear that, even
170	considering ten dimensions in the multivariate space of relative warps, the transitional specimens
171	do not occupy a definite cluster. These results do not change restricting the parallel coordinates
172	plot to the quartiles of each livery - that is, considering only the main portions of morphospace
173	occupied by each colour phase – as shown in Supplementary Fig. S2.
174	Convex hull volume computations revealed that 12.37% of the morphospace of occupied by
175	primary and secondary individuals is shared between colour phases.
176	Analyses carried out on a single sampling site and on the subset of geographically close sampling
177	sites gave similar results with significant regression of shape variables on centroid size (Wilk's

178 Lambda 0.13 and p=0.03 for the Mallorca sampling site, Wilk's Lambda 0.19; p<0.001 for the

179 pooled Sicilian sampling sites) and plots of relative warps and relative warps in size-shape space

180 showing a certain degree of overlap between colour phases (data not shown).

181

# 182 **Discussion**

183 In all the plots of relative warps and relative warps in size-shape space it can be noticed that, while 184 in general primary and secondary specimens occupied different portions of multivariate spaces, 185 there was also a certain degree of overlap (12.37% of convex hull volume). This means that there 186 are typical "feminine" (primary) and "masculine" traits of body shape but not all the specimens of 187 a certain livery conform to this model (that is, there are primary individuals with secondary traits 188 and vice versa). It is also remarkable that in both relative warps and relative warps in size-shape 189 space transitional individuals do not form a definite cluster. While the absence of a definite critical 190 size for colour change - evidenced in our analysis by the overlap of primary, transitional and 191 secondary specimens along the first axis in size-shape space - has already been highlighted in 192 Coris julis (Bentivegna and Cirino 1984), the overlap in body shape is a new finding. Bentivegna 193 and Cirino (1984) proposed that in C. julis there could be individuals that always retain the female 194 phase and showed that sexual inversion can be triggered by social factors (male/female ratio). 195 Social control of sex change is considered common in species with haremic social systems in 196 which dominant males control access to females (Muñoz and Warner 2004). In the light of 197 Bentivegna and Cirino's (1984) observations, our results suggest that shape changes simply 198 happen during growth without regard to colour phase and that livery changes can happen at most 199 of growth stages without interfering with changes in body shape. So, if at a certain point during 200 growth the conditions for livery (sexual) change are present, the individual changes its colour, if 201 not it continues to grow changing its shape without changing its colouration. 202 However, given that specimens with secondary colour pattern are territorial and engage in 203 agonistic interactions with each other (Lejeune 1982; Bentivegna and Cirino 1984), and given that 204 the species is protogynous, most larger specimens are secondary while very large primary 205 specimens are rare. This is in agreement with a size-advantage hypothesis of sex change. In fact, it 206 has been noticed in other protogynous species that the females mate chiefly with larger, older

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207 males and this factor of strong sexual selection often leads to sexual dimorphism, with males 208 developing specialized structures and colourations for combat and display (Warner and Robertson 209 1978). A positive correlation of size with reproductive success has been noticed in Halichoeres 210 melanurus males, where it has been hypothesized that size is an important trait in male-male 211 territorial competition while male colouration is important for female mate choice (Kuwamura et 212 al. 2000). 213 Our study also highlights that larger specimens (which are usually secondary in colouration and 214 males) have a deeper body and a steeper head profile, a situation which resembles what happens in 215 other species. In fact, males of many kinds of bony fishes have a prominent nuchal hump on top of 216 the head (Barlow and Siri 1997). In particular, such a nuchal hump has been studied in neotropical 217 cichlids and various hypotheses have been tested to explain this trait (Barlow 1998). However, 218 while Barlow (1998) suggests that in Amphilophus citrinellus the presence of a nuchal hump can 219 be a means of sex recognition, this hardly applies to C. *julis* due to the striking difference in colour 220 patterns between primary and secondary specimens in this species. Here, it is hypothesized that the 221 deeper body and steeper head profile accompany the striking secondary colour pattern, enhancing 222 it (the vivid colours are probably more visible in a bigger and deeper body) for social display 223 purposes (for instance in mate choice or in agonistic relationships between secondary males). It is 224 worth noticing that in *Thalassoma bifasciatum* terminal males body depth is positively correlated 225 with the success in defending territories from smaller males (Warner and Schultz 1992). A survey 226 of pictures and drawings (Randall and Kuiter 1982; Gomon and Randall 1984; Randall unpubl 227 data) of other Coris species also reveals that a deeper body with growth/terminal livery is easily 228 recognizable in Coris aygula, C. ballieui, C. bulbifrons, C. cuvieri, C. debueni, C. formosa, C. 229 gaimard, C. hewetti and C. sandeyeri. Coris aygula and C. bulbifrons also develop a nuchal hump 230 similar to the one found in cichlid species. These findings suggest that the pattern described here is 231 common to other labrid species of the genus and might represent a new avenue for research on 232 sequentially hermaphroditic fish species. In fact, it is possible that the pronounced difference in 233 colour between primary and secondary specimens observed in C. julis allows the observed 234 decoupling of body shape and colour phase, leaving body shape to be a useful but not strictly 235 necessary "enhancer" of the function of colour. Alternatively, body shape might be more important 236 in species where chromatic differences between liveries are less pronounced; in these cases, colour 237 and body shape changes during growth might be more strictly linked. The relative importance of

238 colouration and body shape in aggressive and/or display behaviour of secondary individuals will

- 239 need to be more thoroughly tested in *C. julis* and similar species.
- 240 Conclusions
- 241 *C. julis* shows a significant allometric variation in body shape which results in deeper bodies in
- 242 larger individuals. While on average secondary individuals possess a deeper body compared to
- 243 primary individuals a common occurrence also in other species of the genus body shape in C.
- 244 *julis* changes during growth without being constrained by colour phase. For this reason, it can be
- argued that secondary individuals have, on average, a deeper body, just because they are larger and
- that the hormonal changes which result in sex and colour change seem not to have effect on body
- shape.
- 248 Finally, our study also shows that parallel coordinate plots can be a useful exploratory tool when
- 249 dealing with geometric morphometric data.
- 250

### 251 Acknowledgements

- 252 The authors would like to acknowledge the precious contribution of F. James Rohlf who provided
- 253 many useful suggestions at multiple stages. Enrico Arneri, Ferdinando Boero, G. Andrea de Lucia,
- 254 Paolo Domenici, Maria Ghelia, Paolo Guidetti, Miquel Palmer, Sergio Ragonese, Alfonso Toscano
- and Nedo Vrgoc kindly helped in obtaining specimens used in the analysis.
- 256

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### 353

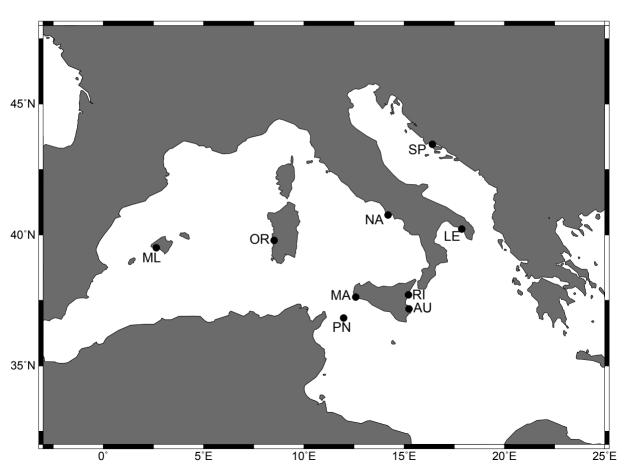
### **Figure captions** 354

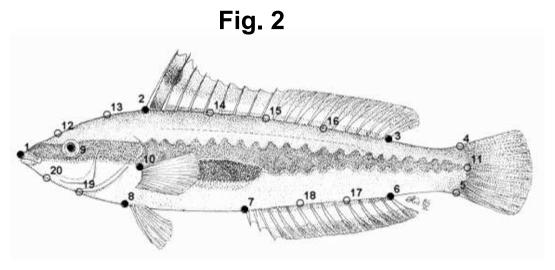
355	Fig. 1 Geographic locations for the samples used in the study. AU=Augusta, SR, Italy; LE=Porto
356	Cesareo, LE, Italy; ML=Badia de Palma, Mallorca, Spain; MA=Mazara del Vallo, TP, Italy;
357	NA=Capo Posillipo, Naples, Italy; OR=Oristano, Italy; PN=Pantelleria, Italy; RI=Riposto, CT,
358	Italy; SP=Split, Croatia
359	
360	Fig. 2 Position of the points used for the analysis of body shape. Filled circles represent
361	landmarks, open circles semilandmarks
362	
363	Fig. 3 Change in shape associated with change in size represented with a "wireframe graph"
364	produced by the software MorphoJ. The dashed grey line represents a reference shape, the black
365	solid line represents the change in shape relative to the reference as the body size increases
366	
367	Fig. 4 Three-dimensional scatterplot of the first three relative warps (RW) in size-shape space;
368	the scale of the axes has been distorted deliberately to visualize the patterns on RW2 and RW3.
369	The plot shows a considerable overlap of primary and secondary individuals, as well as the lack of
370	a definite region for transitional specimens
371	
372	Supplementary Fig. S1 Parallel coordinate plot of the first ten relative warps for all the studied
373	specimens. Yellow=primary, blue=transition, pink=secondary. The plot shows a considerable
374	overlap of the morphospace portions occupied by each colour phase
375	
376	Supplementary Fig. S2 Parallel coordinate plot of the first ten relative warps. For each colour
377	phase only the quartiles are shown to highlight the degree of overlap among groups.
378	Yellow=primary, blue=transition, pink=secondary. The plot shows that the overlap in
379	morphospace occupation of primary, transitional and secondary individuals is not caused by a few
380	outliers but it is evident even considering only the quartiles of the relative warps of each colour
381	phase
382	

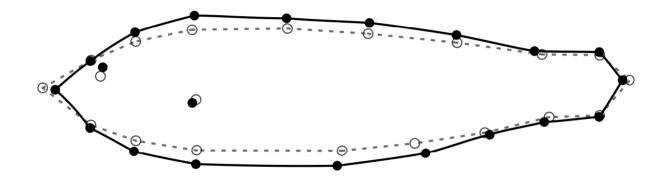
Contral	Commission disc	e Environment Coordi	Coordinate		Livery		
Sample	Sampling time		Coordinates	Primary	Transition	Secondary	Total
Split (SP)	March 2007	Posidonia oceanica beds; a few meters of depth	43°28'N 16°24'E	7	9	10	26
Naples (NA)	February 2007	Gravel bottom; 10- 12 meters of depth	40°46'N 14°12'E	4	21	8	33
Lecce (LE)	May 2007 and May 2009	Patches of rocky bottom and <i>Posidonia oceanica</i> beds; 8-20 meters of depth	40°14'N 17°52'E	3	1	29	33
Oristano (OR)	April 2007	Rocky bottom with photophilic algae; <i>Posidonia oceanica</i> beds; 5-6 meters of depth	39°48'N 8°31'E	24	4	2	30
Mallorca (ML)	June 2007	Rocky bottom with photophilic algae; 9- 17 meters of depth	39°31'N 2°39'E	21	2	19	42
Riposto (RI)	January 2007; March 2007; April 2008	Patches of rocky and sandy bottom; 4-8 meters of depth	37°43'N 15°13'E	26	-	2	28
Mazara del Vallo (MA)	June 2007	Posidonia oceanica beds; 3-5 meters of depth	37°38'N 12°35'E	14	2	19	35
Augusta (AU)	May 2008 and June 2008	Rocky bottom with few photophilic algae; 4-10 meters of depth	37°11'N 15°14'E	6	-	17	23
Pantelleria (PN)	October 2008	Rocks with Posidonia oceanica patches close to a sandy area with Posidonia; 5-15 meters of depth	36°50'N 11°59'E	1	1	11	13
Total				106	40	117	263

# Table 1 Sampling data for each population used in the study

# Fig. 1

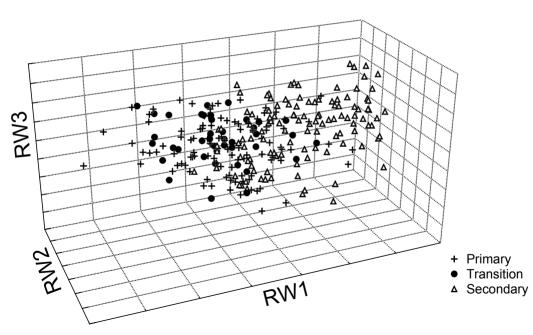


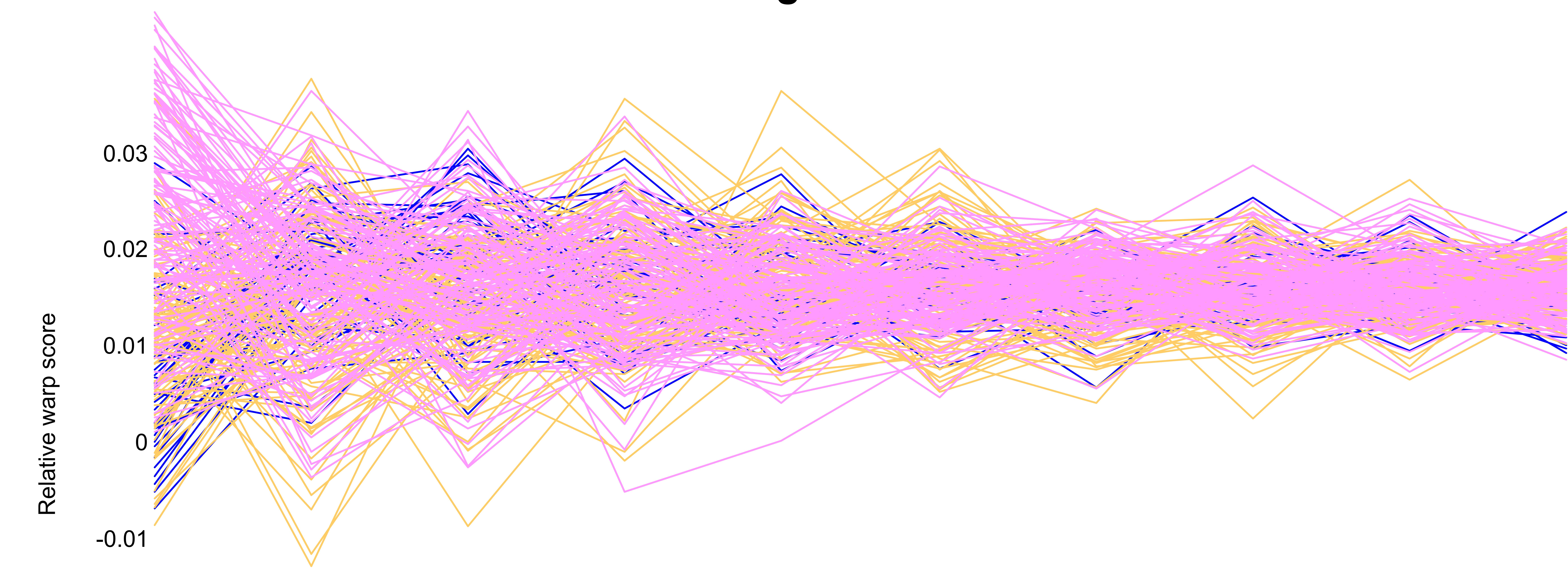






# Fig. 4





-0.02

-0.03

2

# Fig. S1

6 Relative warp



