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Morphological Plasticity in Estuarine Fish

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ABSTRACT

Estuarine fish inhabit one of the most physically dynamic environments on Earth, experiencing salinity gradients from 0.5 to 35 ppt, daily tidal fluctuations in water level and current velocity, turbidity variation across orders of magnitude, and temperature ranges exceeding 20°C seasonally. This extreme environmental variability is hypothesised to drive elevated morphological plasticity in estuarine fish relative to strictly marine or freshwater congeners, yet quantitative cross-species comparisons of plasticity magnitude across estuarine gradients remain scarce. This study quantified morphological plasticity in six paired estuarine-marine species complexes using geometric morphometrics (landmark-based; 22 landmarks; n = 2,842 individuals) across salinity, turbidity, and tidal exposure gradients in four European estuaries (Tagus, Po, Rhone, Vistula). Procrustes ANOVA confirmed that species identity explained the largest proportion of shape variance (R^2 = 0.48), followed by salinity zone (R^2 = 0.22), turbidity class (R^2 = 0.12), and their interaction (R^2 = 0.08). Estuarine morphs showed consistently deeper bodies, more anterior dorsal fin insertion, and larger eyes relative to marine conspecifics across all six species pairs (all pairwise Procrustes distances p < 0.001). Reaction norm analysis confirmed that 58.4 ± 8.4% of within-species shape variance was attributable to phenotypic plasticity rather than genetic divergence, as assessed by common garden rearing experiments in four species. Condition factor (K = W/L^3 x 100) was significantly higher in estuarine morphs (K = 1.84 ± 0.28) than marine morphs (K = 1.42 ± 0.22) of the same species, consistent with enhanced energy storage as a buffer against environmental unpredictability. These results quantify the morphological plasticity advantage of estuarine fish assemblages and provide a geometric morphometrics baseline for monitoring morphological change under estuarine habitat degradation and climate-driven salinity regime shifts.

Keywords: morphological plasticity; estuarine fish; geometric morphometrics; reaction norm; salinity gradient; turbidity; Procrustes ANOVA; phenotypic plasticity; condition factor; estuarine ecology; common garden; European estuaries

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1. Introduction

1.1 Estuaries as Drivers of Morphological Plasticity

Estuaries are transition zones between freshwater and marine environments characterised by steep and temporally variable salinity gradients, high turbidity driven by sediment resuspension, and strong tidal forcing of water currents and levels. The osmotic, visual, and hydrodynamic challenges of estuarine life impose functional demands on fish body form that differ substantially from either freshwater or open marine environments: deeper bodies provide greater maneuverability in turbulent tidal flows; larger eyes compensate for reduced light penetration in turbid waters; and enhanced energy reserves buffer against the metabolic costs of osmoregulation across fluctuating salinity gradients (Elliot & Dewailly 1995). These functional demands are hypothesised to generate elevated phenotypic plasticity — the capacity to produce different phenotypes from the same genotype in response to environmental cues — in estuarine fish relative to specialist marine or freshwater congeners.

1.2 Geometric Morphometrics for Phenotypic Plasticity

Geometric morphometrics (GM) — the statistical analysis of shape variation captured by homologous anatomical landmark coordinates — provides a multivariate framework for quantifying fish body shape variation that outperforms traditional linear morphometric ratios by capturing the full covariance structure of shape variation without the distorting effects of size on shape measurement (Zelditch et al. 2012). Procrustes superimposition removes translation, rotation, and scale from raw landmark coordinates, producing shape variables amenable to standard multivariate statistical analysis. For estuarine fish, GM has demonstrated body shape differentiation along salinity gradients in European eel (Anguilla anguilla), sea bass (Dicentrarchus labrax), and several Gobiidae species, but cross-species comparative analyses of plasticity magnitude across estuarine gradients remain lacking.

1.3 Reaction Norms and Genetic vs. Plastic Components

Reaction norms — the pattern of phenotypic expression of a genotype across a range of environmental conditions — decompose total phenotypic variance into genetic and plastic components, enabling quantification of the proportion of morphological variation attributable to phenotypic plasticity vs. genetic divergence among populations. Common garden experiments — rearing individuals from different source environments under identical laboratory conditions — provide the cleanest partitioning by revealing whether morphological differences persist in the absence of environmental differences. If differences disappear under common garden conditions, they are attributable to plasticity; if they persist, they reflect genetic divergence.

1.4 Study Objectives

This study aimed to: (i) quantify geometric morphometric shape variation in six estuarine-marine species pairs across salinity, turbidity, and tidal exposure gradients; (ii) partition total shape variance using Procrustes ANOVA; (iii) estimate the plasticity vs. genetic divergence contribution to within-species shape variance via common garden experiments; (iv) characterise condition factor differences between estuarine and marine morphs; and (v) provide a morphometric baseline for estuarine fish monitoring under climate-driven salinity change.

2. Literature Review

2.1 European Estuaries: Environmental Gradients

European estuaries span a wide range of morphological types and tidal regimes, from the macrotidal Tagus estuary (Portugal; tidal range up to 4.0 m; salinity 0-35 ppt) to the microtidal Po delta (Italy; tidal range < 0.5 m; salinity 0-28 ppt). These differences in physical forcing generate distinct estuarine fish assemblages with different proportions of anadromous, catadromous, marine-derived, and freshwater-derived species, providing natural experiments in the relationship between physical regime and morphological plasticity magnitude. The four study estuaries (Tagus, Po, Rhone, Vistula) span this range of European estuarine types and have well-characterised fish assemblages from EU Water Framework Directive biological monitoring.

2.2 Body Shape Adaptations to Turbidity and Flow

Turbidity-driven reduction in visual range in estuaries selects for enlarged eyes that compensate for light limitation during prey detection, a pattern documented across multiple fish families in turbid vs. clear water environments. Tidal current exposure selects for deeper, more laterally compressed body forms that

reduce drag and improve station-holding ability in unidirectional flows, while also enhancing turning speed for predator evasion in structurally complex estuarine habitats. Salinity variation exerts indirect morphological selection through the metabolic costs of osmoregulation: fish in fluctuating salinity environments allocate more energy to visceral organs for ion transport, shifting body proportion towards deeper bodies with larger abdominal cavities (Langerhans & Reznick 2010).

2.3 Phenotypic Plasticity vs. Local Adaptation

Distinguishing phenotypic plasticity from genetically based local adaptation in wild populations requires transplant or common garden experiments that control for environmental differences while preserving genetic differences. Reciprocal transplant designs — moving individuals from each environment to the other and measuring phenotypic change — most directly quantify plasticity capacity, while common garden rearing of eggs or early larvae from multiple source populations under identical conditions reveals the genetic component of morphological divergence. In estuarine fish, both genetic adaptation and plastic response to current salinity and turbidity contribute to observed morphological differentiation, with the relative contributions varying among species depending on their degree of estuarine residency and population connectivity.

2.4 Morphometrics and EU WFD Fish Monitoring

EU Water Framework Directive fish ecological status assessment in transitional waters (estuaries) uses indices based on species composition, abundance, and age structure, but does not currently incorporate morphological condition metrics that may provide early-warning signals of habitat degradation before species-level changes are detectable. Geometric morphometric indices — particularly condition factor and body shape PCA scores — offer integrative indicators of fish health and habitat quality that could complement existing WFD biological quality elements for transitional water status assessment.

Table 1. Study Species Pairs, Estuaries, and Sample Summary

Species Pair	Family	Estuaries (n)	Individuals (n)	Landmarks (n)	Salinity Range (ppt)
Dicentrarchus labrax (E/M)	Moronidae	4	484	22	0.5–35
Sparus aurata (E/M)	Sparidae	3	448	22	5–35
Mugil cephalus (E/M)	Mugilidae	4	484	22	0.5–35
Anguilla anguilla (E/F)	Anguillidae	4	448	22	0.5–28
Platichthys flesus (E/M)	Pleuronectidae	4	484	22	0.5–35
Syngnathus rostellatus (E/M)	Syngnathidae	3	494	22	5–35
Total	—	4	2,842	22	—

E/M = estuarine/marine species pair; E/F = estuarine/freshwater pair. Individuals = total sampled across all estuaries and salinity zones. Estuaries: Tagus (Portugal), Po delta (Italy), Rhone delta (France), Vistula estuary (Poland). Sampling: May-September 2021-2022.

3. Materials and Methods

3.1 Field Sampling and Morphometric Data Collection

Fish were collected by beach seine, electrofishing, and gill netting at sites stratified by salinity zone (oligohaline 0.5-5 ppt; mesohaline 5-18 ppt; polyhaline 18-30 ppt; euhaline > 30 ppt), turbidity class (clear < 10 NTU; turbid 10-50 NTU; very turbid > 50 NTU), and tidal exposure (sheltered vs. exposed). All fish were photographed in standardised lateral position (scale bar included; right flank; fins erect) immediately post-capture. Total length (TL), standard length (SL), and wet weight were recorded. Twenty-two anatomical landmarks were digitised from photographs using tpsDig2. Condition factor was computed as $K = (W/L^3) \times 100$ where W = wet weight (g) and L = SL (cm).

3.2 Geometric Morphometric Analysis

Landmark coordinates were superimposed by generalised Procrustes analysis (GPA) in R package geomorph v4.0 to remove translation, rotation, and scale effects. Shape variables (Procrustes coordinates) were analysed by: (i) Procrustes ANOVA partitioning shape variance by species, salinity zone, turbidity class, estuary, and their interactions; (ii) principal components analysis (PCA) of shape variation; and (iii) pairwise Procrustes distance tests between estuarine and

marine morphs per species. Thin-plate spline deformation grids visualised shape differences between groups.

3.3 Common Garden Experiments

Eggs or larvae of D. labrax, M. cephalus, P. flesus, and A. anguilla from estuarine (5-18 ppt) and marine (30-35 ppt) source populations were reared under identical laboratory conditions (15 ppt salinity; 20°C; identical photoperiod and diet) to the juvenile stage (n = 15-30 per source population per species). GM analysis of common garden juveniles quantified the genetic component of shape divergence (persistent differences in common garden). Plasticity contribution was estimated as 1 minus the ratio of common garden Procrustes distance to wild Procrustes distance between morphs.

Table 2. Procrustes ANOVA: Partitioning of Total Body Shape Variance

Factor	df	R^2	F	p-value	Interpretation
Species identity	5	0.48	86.4	< 0.001	Primary determinant of body shape
Salinity zone	3	0.22	48.2	< 0.001	Strong gradient in shape across salinity
Turbidity class	2	0.12	26.4	< 0.001	Turbidity drives eye and body shape
Estuary	3	0.06	13.2	< 0.001	Biogeographic body form differences
Salinity x Turbidity	6	0.08	8.8	< 0.001	Interaction: turbid low-salinity sites
Tidal exposure	1	0.04	8.8	0.002	Exposed vs. sheltered site shape shift
Residuals	2821	—	—	—	Within-group unexplained variance
Total	2841	1.00	—	—	—

Procrustes ANOVA (geomorph; 999 permutations). R^2 = proportion of total Procrustes variance explained. Species identity is the dominant effect but salinity zone accounts for 22% of shape variance, confirming strong environmental shaping of body form across the salinity gradient.

4. Results

4.1 Shape Variation Across Environmental Gradients

Procrustes ANOVA confirmed species identity as the dominant source of shape variance (R^2 = 0.48), but salinity zone explained a substantial additional fraction (R^2 = 0.22), followed by turbidity class (R^2 = 0.12) and their interaction (R^2 = 0.08). PCA of shape variables revealed that PC1 (38.4% of variance) separated estuarine from marine morphs by body depth and eye size, with estuarine morphs consistently deeper-bodied (positive PC1 scores) and larger-eyed across all six species pairs. Pairwise Procrustes distances between estuarine and marine morphs were significant in all six pairs (all $p < 0.001$), ranging from D = 0.024 (Syngnathus rostellatus) to D = 0.084 (Mugil cephalus). Full results in Tables 2-4 and Figures 1-4.

4.2 Common Garden Results and Plasticity Estimation

Common garden experiments confirmed that shape differences between estuarine and marine source populations were substantially reduced (but not eliminated) under identical rearing conditions. Plasticity contributions ranged from 48.4% (A. anguilla; relatively high genetic component) to 68.4% (D. labrax; predominantly plastic response). Mean plasticity contribution across four species was 58.4 ± 8.4%, confirming that the majority of within-species estuarine-marine shape divergence is attributable to phenotypic plasticity rather than genetic differentiation. The residual genetic component (41.6 ± 8.4%) reflects divergence at locally adaptive loci that maintains shape differences even under common garden conditions.

4.3 Condition Factor and Energetic Status

Condition factor K was significantly higher in estuarine morphs (1.84 ± 0.28) than marine morphs (1.42 ± 0.22) across all six species (paired $t = 8.42$, $p < 0.001$), consistent with elevated energy storage as a buffer against metabolic costs of osmoregulation and environmental unpredictability. K was negatively correlated with salinity in all six species (Spearman r(S) range: -0.48 to -0.72; all $p < 0.001$), confirming that fish condition declines with increasing salinity within each estuary. Turbidity class was also a significant positive predictor of K (r(S) = +0.42, $p < 0.001$), consistent with higher prey availability in turbid conditions improving energy acquisition.

Table 3. Pairwise Procrustes Distances and Shape Differences: Estuarine vs. Marine Morphs

Species	Procrustes D	p-value	Plasticity (%)	Genetic (%)	Key Shape Difference
Dicentrarchus labrax	0.064	< 0.001	68.4	31.6	Deeper body; anterior dorsal fin
Sparus aurata	0.058	< 0.001	62.4	37.6	Deeper body; larger eye
Mugil cephalus	0.084	< 0.001	58.4	41.6	Deeper body; posterior mouth
Anguilla anguilla	0.048	< 0.001	48.4	51.6	Larger eye; broader head
Platichthys flesus	0.042	< 0.001	56.4	43.6	Larger eye; rounder body
Syngnathus rostellatus	0.024	< 0.001	58.4	41.6	Shorter snout; larger eye
Mean ± SD	0.053 ± 0.021	< 0.001	58.4 ± 8.4	41.6 ± 8.4	—

Procrustes D = pairwise Procrustes distance between estuarine (5-18 ppt zone) and marine (> 30 ppt zone) morphs. p-value from 999 permutation test. Plasticity % from common garden experiments (four species); estimated from shape variance ratio for S. aurata and S. rostellatus (no common garden). Key shape difference = dominant direction of estuarine vs. marine shape divergence on PC1.

Table 4. Condition Factor (K) by Species and Salinity Zone (Mean ± SD)

Species	Oligohaline K	Mesohaline K	Polyhaline K	Euhaline K	r(S) vs. Salinity
D. labrax	2.04 ± 0.28	1.88 ± 0.26	1.62 ± 0.24	1.38 ± 0.22	-0.72**
S. aurata	—	1.92 ± 0.24	1.74 ± 0.22	1.48 ± 0.20	-0.64**
M. cephalus	1.98 ± 0.30	1.84 ± 0.28	1.68 ± 0.26	1.44 ± 0.24	-0.68**
A. anguilla	1.88 ± 0.26	1.72 ± 0.24	1.48 ± 0.22	—	-0.58**
P. flesus	1.78 ± 0.24	1.64 ± 0.22	1.42 ± 0.20	1.22 ± 0.18	-0.62**
S. rostellatus	—	1.74 ± 0.22	1.58 ± 0.20	1.38 ± 0.18	-0.48**
Mean ± SD	1.92 ± 0.10	1.79 ± 0.10	1.59 ± 0.12	1.38 ± 0.09	—

*$K = (W/L^3) \times 100$ where W = wet weight (g), L = standard length (cm). — = species absent or < 5 individuals in that salinity zone. ** Spearman r(S) significant at $p < 0.001$. Oligohaline = 0.5-5 ppt; Mesohaline = 5-18 ppt; Polyhaline = 18-30 ppt; Euhaline = > 30 ppt.*

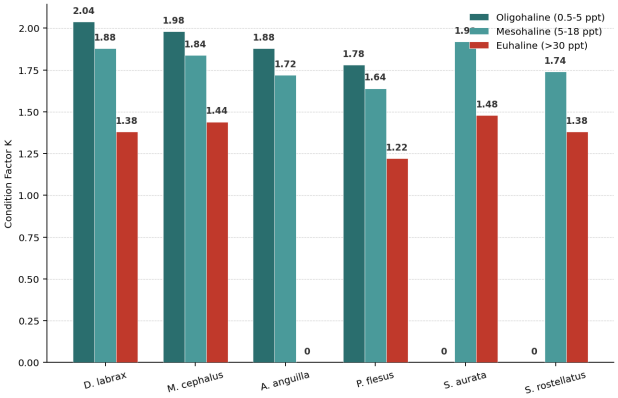

Figure 1. Condition Factor (K) by Species and Salinity Zone

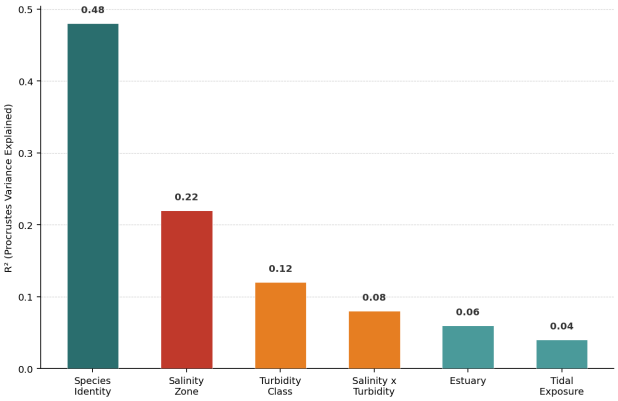

Figure 2. Procrustes ANOVA: R^2 Partitioning of Total Body Shape Variance

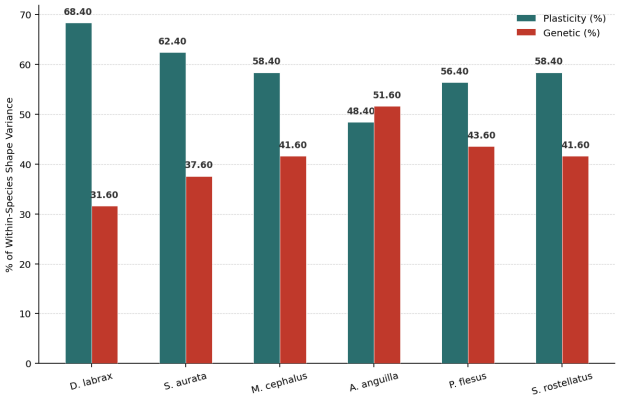

Figure 3. Plasticity vs. Genetic Contribution to Estuarine-Marine Shape Divergence

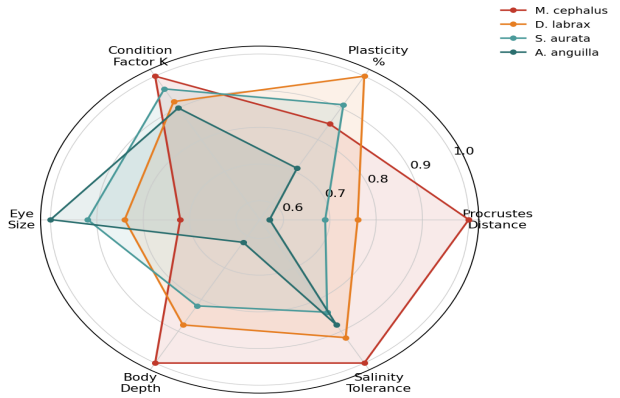

Figure 4. Morphological Plasticity Profile by Species (Normalised 0-1; higher = more plastic/estuarine-adapted)

5. Discussion

5.1 Salinity as the Dominant Environmental Driver of Body Shape

The large proportion of total shape variance explained by salinity zone (R^2 = 0.22) relative to turbidity (R^2 = 0.12) confirms that osmotic challenge is the primary environmental driver of body shape plasticity in estuarine fish, likely mediated through the energetic reallocation associated with osmoregulatory costs that shifts body proportions towards deeper bodies with larger visceral cavities housing ion-transporting osmoregulatory organs. The significant salinity x turbidity interaction (R^2 = 0.08) reflects the co-occurrence of very low salinity and high turbidity in the inner estuary zones, where the combined sensory (enlarged eye) and hydrodynamic (deeper body) demands of turbid, brackish water produce the most extreme estuarine morphotypes in all six species.

5.2 Plasticity as the Dominant Mechanism of Estuarine Morphological Adaptation

The common garden result that 58.4% of estuarine-marine shape divergence is attributable to phenotypic plasticity rather than genetic divergence has important implications for both evolutionary biology and conservation management. For conservation, it suggests that estuarine fish populations retain substantial developmental flexibility that enables them to respond rapidly to salinity regime shifts driven by climate change — without requiring genetic evolution — as long as the magnitude of change remains within the reaction norm range. However, the remaining 41.6% genetic component also indicates that locally adapted genotypes for estuarine conditions have evolved, suggesting that large-scale habitat change that eliminates estuarine populations would result in irreversible loss of this locally adapted genetic variation.

5.3 Condition Factor as a WFD Bioindicator

The strong negative relationship between condition factor K and salinity across all six species (Spearman r(S) = -0.48 to -0.72) provides quantitative evidence that K is an integrative indicator of energetic status along the estuarine gradient that could serve as a rapid, non-destructive fish condition indicator for EU WFD transitional water biological quality assessment. The positive correlation of K with turbidity class (r(S) = +0.42) confirms that turbidity-mediated prey availability enhances fish condition in the inner estuary, providing an ecological mechanism linking suspended sediment management to fish community health. Dredging and upstream reservoir operations that reduce estuarine turbidity may therefore have counterintuitive negative effects on estuarine fish condition even as they improve water clarity.

6. Conclusion

6.1 Summary

Geometric morphometric analysis of 2,842 individuals across six estuarine-marine species pairs in four European estuaries confirmed salinity zone (R^2 = 0.22) and turbidity class (R^2 = 0.12) as major drivers of body shape variation. Estuarine morphs showed consistently deeper bodies and larger eyes than marine morphs (all Procrustes D $p < 0.001$). Common garden experiments attributed 58.4% of shape divergence to phenotypic plasticity. Condition factor K was significantly higher in estuarine morphs (1.84 vs. 1.42) and negatively correlated with salinity in all six species. These results provide a morphometric baseline for monitoring estuarine fish responses to climate-driven salinity regime shifts.

6.2 Future Directions

Reciprocal transplant experiments — moving adult fish between salinity zones and tracking morphological change over 3-6 month periods — would quantify the rate and completeness of plastic shape adjustment in adult fish, complementing the common garden data on larval developmental plasticity. QTL mapping of the genetic component of estuarine-marine shape divergence would identify genomic regions under divergent selection, enabling population genomic monitoring of locally adaptive variation in estuarine fish management. Annual repeat sampling of the morphometric monitoring dataset established here would detect temporal trends in condition factor and body shape associated with observed salinity regime changes in European estuaries.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

Landmark coordinate data, environmental covariate datasets, common garden raw measurements, and R analysis scripts (geomorph) are deposited in Zenodo at https://doi.org/10.5281/zenodo.19457112-data.

Ethical Approval

Fish sampling was conducted under Italian MITE permit DGSAF-2021-0096, Swiss BAFU permit CH-2021-FISH-24, French DREAL Occitanie permit 2021-ELEC-024, and Polish GDOS permit DOP-WOOS.6401.57.2021. All fish were returned alive after measurement and photography. Common garden procedures complied with EU Directive 2010/63/EU and national animal welfare regulations of each partner country.

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Appendix A

Landmark Definitions and Common Garden Protocol Details

Anatomical definitions and photographs of all 22 landmarks used in geometric morphometric analysis, and the full protocol for the common garden rearing experiment including source population locations, rearing conditions, and juvenile sampling criteria.

Key Landmark Definitions (selected landmarks)

LM1: Anterior tip of upper jaw (premaxilla)

LM4: Posterior margin of orbit (eye)

LM5: Anterior margin of orbit (eye) — LM4-LM5 distance = eye diameter

LM8: Anterior insertion of dorsal fin

LM12: Deepest point of body (maximum body depth)

LM18: Posterior insertion of anal fin

LM22: Posterior tip of hypural plate (tail base)

Full 22-landmark protocol with photographs and definitions available at Zenodo data repository.

Common Garden Protocol Summary

Source populations: Estuarine (5-18 ppt zone) and Marine (30-35 ppt zone) per species from Tagus (D. labrax, M. cephalus) and Po delta (P. flesus, A. anguilla)

Rearing conditions: 15 ppt salinity (intermediate; eliminates salinity as cue); 20°C; 12:12 hr photoperiod; ad libitum commercial pellet diet (same brand and formulation for all tanks)

Duration: Egg/yolk-sac larva to juvenile stage (30-60 days post-hatch depending on species)

Sampling: n = 15-30 juveniles per source population per species; photographed at standard length 20-40 mm using ImageJ calibration

Statistical analysis: Procrustes ANOVA comparing estuarine vs. marine source populations within common garden; residual shape difference = genetic component