

Sex differentiation of the European eel in brackish and freshwater environments: a comparative analysis

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Body growth parameters, age and total length at sex differentiation were compared in three European eel *Anguilla anguilla* populations from Mediterranean sites with different salinity. Whilst body growth was faster in brackish than in freshwater environments, the present analysis shows that body size at sex differentiation might be a physiological invariant.

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The European eel *Anguilla anguilla* (L.) is a secondary gonochoristic species (Devlin & Nagahama, 2002) characterized by delayed sex differentiation and metagametic sex determination. Gonad differentiation and development are probably triggered by reaching a certain body size rather than age (Bieniarz *et al.*, 1981; Colombo *et al.*, 1984). Sex determination in *Anguilla* species is not univocally determined by genes, but is presumably influenced by environmental and social factors, with high temperatures and high densities biasing sex ratio towards males (Beullens *et al.*, 1997; Krueger & Oliveira, 1999; Oliveira & McCleave, 2002). Anguillids also show clear sexual dimorphism (Krueger & Oliveira, 1999), with females growing faster and attaining greater body size than males (although this last statement has been recently questioned; Holmgren & Mosegaard, 1996; Holmgren *et al.*, 1997). Body growth shows extreme variability at different spatial scales, from interindividual variation within the same population to geographical variation among different habitats (Vøllestad, 1992; Panfili *et al.*, 1994; De Leo & Gatto, 1995).

The aim of the present study was to use mathematical modelling to test the invariance of sex differentiation and body growth parameters of the European

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eel in different environmental conditions. Although mathematical models have already been used to describe European eel growth (De Leo & Gatto, 1995; Poole & Reynolds, 1996), so far the only way to investigate sex differentiation has been to perform expensive histological analyses. In the present paper, a body growth model and a flexible calibration procedure described in Melià *et al.* (2006) are used to compare three European eel populations from Mediterranean sites with different salinity and subject to different fishing policies. The model explicitly accounts for sexual dimorphism by introducing three distinct von Bertalanffy growth curves for undifferentiated fish, females and males (Melià *et al.*, 2006). Besides the classical von Bertalanffy growth parameters: L_0 [total length (L_T) at age zero, *i.e.* at metamorphosis from glass eel to elver], k_U , k_F and k_M (Brody coefficients for undifferentiated, females and males), $L_{\infty F}$ and $L_{\infty M}$ (asymptotic mean L_T of females and males), the model has two additional parameters for sexual differentiation, namely L^* (L_T at sex differentiation) and x^* (age at sex differentiation). These two parameters, along with the other six von Bertalanffy growth parameters, are estimated from available data.

The calibration requires data on L_T and sex at different ages, possibly covering the whole age span of the population being investigated. The model was applied to three datasets. The first was collected in the brackish waters of the Impériaux and Vaccarès lagoons (Rhône River delta, southern France). These data were used by Melià *et al.* (2006) to develop the growth model used in this work. A second dataset was collected in the adjacent Fumemorte drainage canal, where salinity is much lower. The third was collected in the Valli di Comacchio lagoons (Po River delta, northern Italy), another brackish environment, and was used by De Leo & Gatto (1995) to develop a demographic model for the Comacchio European eel population. Table I provides a concise overview of the three sites and the corresponding data.

The results of applying the growth model to the three datasets are reported in Table II. Uncertainty associated to parameter estimates and relevant statistics were assessed by stratified bootstrapping of the original data (Efron & Tibshirani, 1986; Melià *et al.*, 2006). The original datasets and the correspond-

TABLE I. Main features of the three study sites and corresponding datasets

	Vaccarès–Impériaux	Fumemorte	Valli di Comacchio
Latitude	43°30' N	43°30' N	44°40' N
Longitude	4°30' E	4°30' E	12°10' E
Salinity	3.8–12.4*	0.9	23–37
Winter temperature (°C)	7	7	2
Summer temperature (°C)	21–22	21–22	24
Sampling period	1997–1998	1988–1990	1974–1975
Sample size	290	287	758
L_T (mm)	66–688	120–685	123–709
Age (years)	0–6	0–11	0–12

Salinity and temperature data from Dallochio *et al.* (1998), Acou *et al.* (2003) and Poizat *et al.* (2004).

*At Vaccarès; salinity is higher and more variable at Impériaux (5.7–38.0; Poizat *et al.*, 2004).

TABLE II. Basic statistics of growth curve parameters. For each parameter, the first row reports mean \pm s.d., whilst the second row shows median and 90% CI (between parentheses). Parameter distributions were obtained by stratified bootstrapping of the original age and length data (1000 iterations)

Parameter	Vaccarès–Impériaux	Fumemorte	Valli di Comacchio
L_0 (mm)	60.01 \pm 0.19 [†] 60.01 (59.71–60.33)	60.01 \pm 0.19 [†] 60.01 (59.71–60.33)	60.0* 60.0
x^* (years)	1.67 \pm 0.35 1.79 (0.87–1.89)	2.16 \pm 0.22 2.18 (1.77–2.42)	1.62 \pm 0.25 1.65 (1.01–1.91)
L^* (mm)	204.34 \pm 38.47 215.15 (114.78–239.56)	209.23 \pm 21.32 217.04 (171.06–235.23)	268.65 \pm 23.86 275.58 (206.52–290.50)
k_U (year ⁻¹)	26.22 \pm 166.23 0.37 (0.03–72.70)	3.55 \pm 3.43 1.42 (0.70–9.46)	19.36 \pm 78.66 1.68 (1.40–142.38)
$L_{\infty F}$ (mm)	579.73 \pm 50.35 573.00 (510.66–667.71)	515.91 \pm 17.84 514.74 (488.88–547.16)	547.61 \pm 4.28 547.47 (540.85–554.67)
k_F (year ⁻¹)	0.63 \pm 0.18 0.61 (0.40–0.97)	0.39 \pm 0.05 0.38 (0.31–0.48)	0.52 \pm 0.05 0.53 (0.45–0.60)
$L_{\infty M}$ (mm)	387.84 \pm 12.77 386.43 (369.76–409.75)	396.27 \pm 13.15 395.00 (379.96–414.35)	429.87 \pm 2.22 429.76 (426.13–433.56)
k_M (year ⁻¹)	1.10 \pm 0.61 1.11 (0.60–1.70)	1.47 \pm 2.33 0.67 (0.44–6.39)	0.86 \pm 2.57 0.75 (0.64–0.93)

[†] L_0 was calculated as the average length of glass eels entering the Camargue water system.

*As no data about glass eels were available for Comacchio, L_0 was *a priori* set equal to the value of the two French sites.

L_0 , total length at age zero; x^* , age at sex differentiation; L^* , total length at sex differentiation; k_U , Brody coefficient for undifferentiated fish; $L_{\infty F}$, asymptotic female total length; k_F , Brody coefficient for females; $L_{\infty M}$, asymptotic male total length; k_M , Brody coefficient for males.

ing fitting curves are shown in Fig. 1. To fit the data, median values of parameters distributions were used instead of means, due to the higher robustness of the median, especially when distributions are strongly skewed.

Females attained a larger L_T (asymptotic body size *c.* 30–50% larger than that of males) and grew faster than males. This is in accordance with other field studies (Vøllestad & Jonsson, 1986; Vøllestad, 1992; Panfili *et al.*, 1994; Poole & Reynolds, 1996), but in contrast with Holmgren & Mosegaard (1996) and Holmgren *et al.* (1997), who claimed that individuals growing faster at the beginning of sex differentiation might develop with higher probability into males. To highlight subtler differences between growth paths of females and males, growth rates were calculated as $dL/dt = k(L_{\infty} - L_T)$ for both sexes at three ages: age at sex differentiation x^* , $x^* + 1$ year and $x^* + 2$ years. Estimates are given in Table III, and confirm faster female growth at all sites (Mann–Whitney *U*-test, $P < 0.001$), except for Fumemorte, where the growth rate of males was higher at age x^* . Although this outcome is consistent with those obtained by Holmgren *et al.* (1997), it should be taken with caution, as male growth at Fumemorte was assessed with a considerably higher level of uncertainty compared to the other sites. In general, growth was more variable in males than in females (as evidenced by s.d. and CI in Table III),

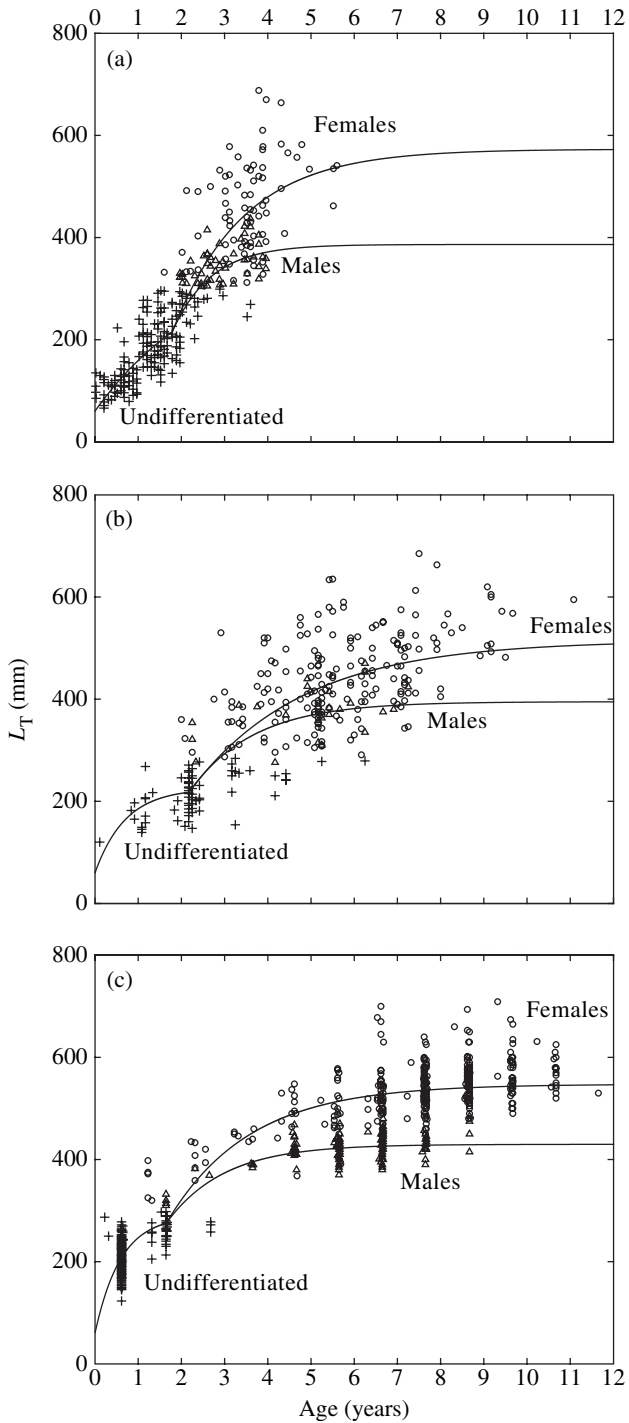


FIG. 1. Growth curves of undifferentiated (+), female (O) and male (Δ) European eels from (a) Vaccarès-Impériaux, (b) Fumemorte and (c) Comacchio. Symbols identify observed age and length data, whilst solid lines show median fitting curves.

TABLE III. Basic statistics of growth rates (mm year⁻¹) as a function of age for females (r_F) and males (r_M). For each parameter, the first row reports mean \pm s.d., whilst the second row shows median and 90% CI (between parentheses)

Parameter	Vaccarès–Impériaux	Fumemorte	Valli di Comacchio
r_F (x^*)	227.93 \pm 31.79	117.64 \pm 10.57	145.23 \pm 10.25
	224.45 (186.53–282.04)	116.68 (102.73–137.41)	144.20 (130.22–162.69)
r_M (x^*)	198.28 \pm 39.48	269.63 \pm 382.43	123.56 \pm 27.87
	192.58 (151.14–266.56)	120.61 (83.48–1203.36)	117.90 (100.19–158.32)
r_F ($x^* + 1$)	120.89 \pm 8.95	79.68 \pm 5.17	86.07 \pm 5.89
	121.64 (107.87–133.58)	78.87 (72.38–89.04)	84.59 (79.56–99.48)
r_M ($x^* + 1$)	64.35 \pm 13.30	54.61 \pm 19.68	56.70 \pm 8.10
	62.58 (45.78–90.52)	57.25 (2.23–79.28)	54.75 (48.99–76.12)
r_F ($x^* + 2$)	65.97 \pm 13.35	54.12 \pm 4.27	51.06 \pm 4.69
	66.99 (41.99–86.15)	53.35 (47.91–61.34)	49.97 (46.07–62.77)
r_M ($x^* + 2$)	23.28 \pm 11.89	26.13 \pm 11.76	26.50 \pm 5.11
	20.37 (8.68–48.73)	29.83 (0.00–38.69)	25.56 (21.57–39.47)

especially in the ages immediately following sex differentiation. Holmgren *et al.* (1997) found scarcely significant differences in L_T increases between the two sexes <300 mm, whilst they observed significantly higher growth rates in females after complete sex differentiation. The assessment of possible links between growth patterns in early developmental stages and sex determination, however, remains an open question and accurate otolith analyses might be very useful to solve it.

Irrespective of their sex, European eels grew faster in brackish than in freshwater environments. This is in accordance with the literature (Panfili *et al.*, 1994; Acou *et al.*, 2003), although the causes are still unclear. Salinity might indeed affect growth rates by influencing food availability, the feeding behaviour or the trophic level at which European eels feed (Edeline & Elie, 2004; Harrod *et al.*, 2005). Asymptotic body size of females was higher in Vaccarès–Impériaux and Comacchio, whilst there was no clear result for males.

Age at sex differentiation was also likely to be influenced by the environment, as it occurred at 20–22 months at the two brackish-water sites (Vaccarès–Impériaux and Comacchio) and at *c.* 26 months at Fumemorte. On the contrary, L^* was almost identical (210–220 mm) in the two Camargue populations (Vaccarès–Impériaux and Fumemorte) and higher (270 mm) at Comacchio. At all sites, however, sex differentiation occurred before macroscopic differentiation became possible (300 mm), in accordance with both histological evidence (Colombo & Grandi, 1996) and the results obtained *via* mathematical models (Melià *et al.*, 2006).

To test for possible invariance of growth and differentiation parameters, empirical bootstrap distributions of all parameters were compared pair-wise (two sites at a time). The L^* was not significantly different at Vaccarès–Impériaux *v.* Fumemorte (Mann–Whitney *U*-test, $P = 0.27$; Wilcoxon matched pairs test, $P = 0.14$). Borderline results were obtained for k_U and k_M at Fumemorte *v.* Comacchio (Mann–Whitney *U*-test, $P < 0.001$ for both parameters; Wilcoxon matched pairs test, $P = 0.20$ and 0.30 , respectively). All other parameter

estimates were significantly different among the three sites ($P < 0.001$ with both tests for all pair-wise comparisons).

The two French populations analysed (Vaccarès–Impériaux and Fumemorte) share the same recruitment. Glass eels must indeed pass through the lagoons, which are connected to the Mediterranean Sea through sluice gates, to enter into the Fumemorte canal. The comparison of sex differentiation parameters between the two populations is therefore of particular interest to highlight the effect of environmental factors on some life-history traits, especially on sexual differentiation. Age at sex differentiation is different between the two sites, whilst L_T is not, as shown in Fig. 2, thus supporting the idea of a critical size as a trigger for sex differentiation (Bieniarz *et al.*, 1981; Colombo *et al.*, 1984). The empirical distribution of x^* and L^* for Comacchio and Vaccarès–Impériaux shows a minor mode at a lower value of both parameters. This might be explained by the fact that some individuals differentiate very early, or indicate a dubious age determination for some European eels, or simply be caused by

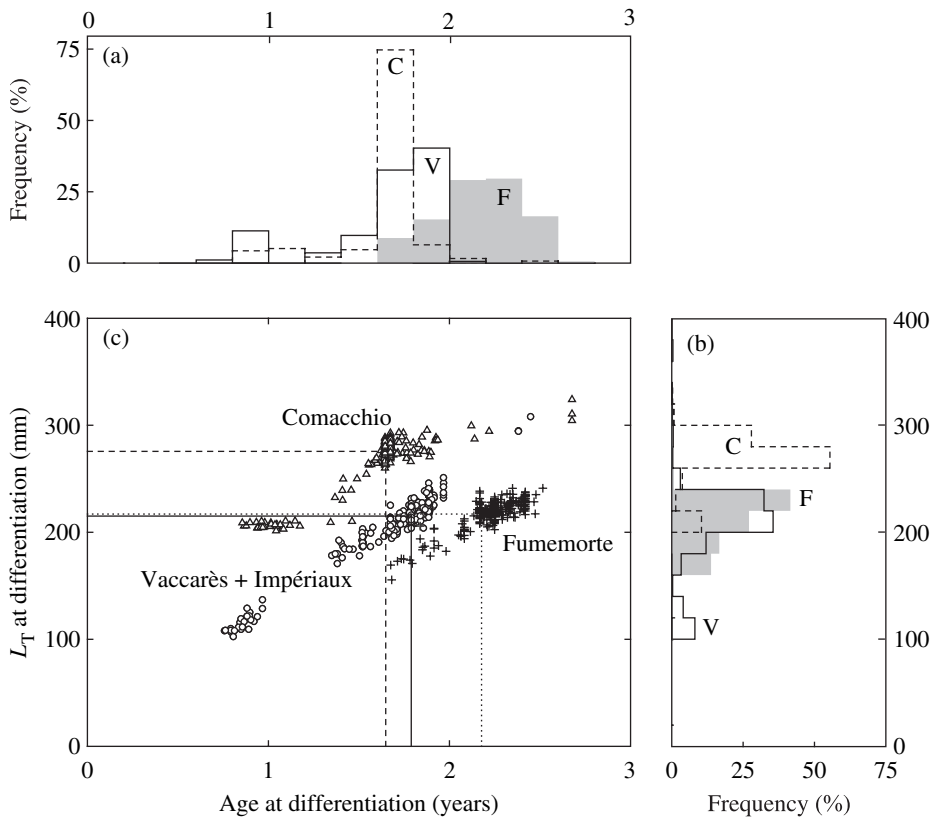


FIG. 2. Bootstrap distributions of age x^* and total length L^* at sex differentiation for populations of European eels. (a) Marginal distribution of x^* , (b) marginal distribution of L^* and (c) joint distribution (each symbol represents a bootstrap replicate, whilst lines show the median point of marginal distributions). ○, — and □: Vaccarès–Impériaux (V); +, ··· and ■: Fumemorte (F); △, ---- and ▭: Comacchio (C).

particularly odd recombinations of data during bootstrap resampling. The different estimate of L^* obtained for Comacchio suggests that this parameter can vary from site to site, at least over a very wide geographical range. It should be noted, however, that the three datasets analysed were collected in different periods. This might have influenced the results if, as suggested by Dannewitz *et al.* (2005), temporal genetic variability in the European eel is as important as (or even the cause of) spatial variability. Also, the three populations are subject to different fishing policies: fishing is indeed not allowed in the Fumemorte canal, whilst it is mainly focused on yellow European eels in the Vaccarès–Impériaux lagoons and concentrated on silver European eels at Comacchio. For this reason, and due to the use of a mesh-size (16 mm) much larger than in Camargue (6 mm), only very few data were available in Comacchio for European eels between 250 and 400 mm. The lack of small fish in the sample might therefore have skewed the estimation of size at sexual differentiation towards larger sizes. A comprehensive comparative analysis based on recent data from different sites would be of great interest to clarify the fascinating issue of determining possible physiological invariants in the European eel, a species characterized by high plasticity of vital rates.

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